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OTHO S. A. SPRAGUE MEMORIAL INSTITUTE

III

COLLECTED REPRINTS

VOLUME X

CHICAGO
1922

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H. GIDEON WELLS, LYDIA M. DEWITT, AND ESMOND R. LONG

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The Inhibitory Action of Certain Organic Mercury Compounds on the Growth of Human Tubercle Bacilli

STUDIES OF THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS. XXII

LYDIA M. DEWITT

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THE INHIBITORY ACTION OF CERTAIN ORGANIC MERCURY COMPOUNDS ON THE GROWTH OF HUMAN TUBERCLE BACILLI

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS. XXII

LYDIA M. DEWITT

From the Otho S. A. Sprague Memorial Institute and the Pathological Laboratory of the University of Chicago

Of all the chemicals so far tested by me during my study of the chemotherapy of tuberculosis, certain organic compounds of mercury have been found the most promising. About a year ago, a report [1] was made of the results so far obtained with about 20 mercury compounds used in experimental tuberculosis and the present report is based on work with 10 organic mercury compounds of phenol, nitro and nitroso phenols and saligenin or phenol carbinol; also on work with about 14 organic mercury compounds of aniline, the nitranilins, and methyl and ethyl anilins and nitranilins. A few other mercury compounds of the benzene nucleus have been used and the results are included in this report. Most of these preparations have been made for me in the Chemical Laboratory of the University of Chicago by Maurice Kharasch, Isadore Jacobsohn and Lyman Chalkley, to all of whom I am indebted for faithfulness and interest.

While my experiments with the organic mercurials were proceeding, Schamberg, Kolmer and Raiziss [2] published a report of a mercurial preparation made by them and called mercurophen or oxy-mercury-ortho-nitro phenolate. This is an interesting compound since it is freely soluble, contains about 53% of mercury, yet is several times as germicidal as mercuric chloride which contains 74% of mercury. Its phenol coefficient for different organisms is from 700 to 3,600 and even 10,000 in some reports. It is nonirritant to the skin, does not tarnish metallic instruments and is much less toxic than mercuric chloride. It was represented as especially valuable for the sterilization of the hands, skin, instruments, tubes, etc.[3] Since some of this compound was sent to me through the kindness of Dr. Schamberg, and since it is of the same type as some of my mercury nitro-phenol compounds, it was tested in the same way and is included in table 1 of this report.

In 1919, Young, White and Schwartz [4] developed an organic mercury compound of the dye fluorescein and called it mercurochrome-220. It contains

Received for publication Oct. 29, 1921.

[1] Jour. Infect. Dis., 1921, 28, p. 150.

[2] Ibid., 1919, 24, p. 547.

[3] Jour. Am. Med. Assn., 1917, 68, p. 1458.

[4] Ibid., 1919, 73, p. 1483. Lancaster, Burnett and Gaus: Ibid., 1920, 75, p. 721. Jour. Urol., 1921, 5, p. 353.

about 26% of mercury, is readily soluble and quite nonirritant and nontoxic. It kills B. coli in 15 minutes in a dilution of 1:5,000 and staphylococcus in a dilution of 1:10,000. It has been used with good effect in washing[4] out infected bladders, in open wounds, sinuses, and in treatment of infections of the throat, nose, ear and eye. Although unlike the other preparations here reported, it is, with mercurochrome 205 and 253, samples of which were also sent me by the kindness of Dr. White, included in table 3 of this report. A preliminary report on mercurophen and mercurochrome 220 in their action on tubercle bacilli and on experimental tuberculosis was made by me about a year ago.[5]

Hirschfelder[6] has made several organic mercury compounds of saligenin or phenol carbinol and has kindly furnished me with quantities sufficient for testing in my work. The results of my tests with these compounds also will be found in table 1.

In all my chemotherapeutic work it has been my custom to test first the power of the chemical being studied to inhibit the growth of the human tubercle bacillus in the test tube, not because we believe that therapeutic value is necessarily closely associated with inhibitory efficiency, but because it is the simplest method of getting a "lead" on the possible value of the compound and the advisability of testing it further. The chemicals which show a high inhibitory power are afterward tested on animals for their bactericidal and therapeutic efficiency. This paper reports only the inhibitory experiments. In every case, human tubercle bacilli of different degrees of virulence have been used, so that many of the tests have been repeated ten or twelve times. The tables give the extremes of variation when the different tests gave different results.

My method has been to add to tubes containing 10 c c of a sterile glycerol agar enough of the compound or of a stock solution of the compound to make dilutions from 1:100 up to 1:1,000,000. The tubes were then well shaken, slanted, cooled, and inoculated; they were examined after 15 days in the incubator, again after 30 days, and sometimes again after 45 days. The highest dilutions on which there was no growth at the end of the longest period allowed is recorded as the limit of "complete inhibition."

The mercury compounds of phenol in this table are all soluble in an alkaline solution so dilute that it does not itself prevent the growth of tubercle bacilli. Phenol itself has been found to inhibit growth of the human tubercle bacillus in a dilution of 1:1,000 but not at 1:5,000. Phenol with HgCl in the ortho position is 100 times as efficient in the

[5] Jour. Am. Med. Assn., 1920, 75, p. 1422.
[6] Jour. Am. Chem. Soc., 1920, 42, p. 2678.

TABLE 1

PHENOLS

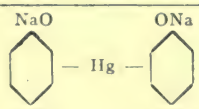
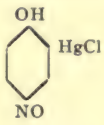
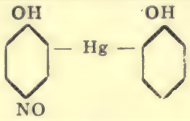
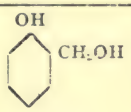
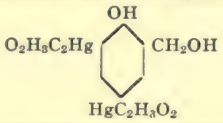
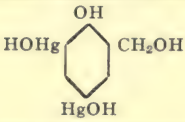
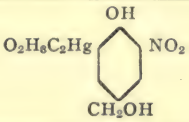
Name	Formula	Percentage of Mercury	Complete Inhibition
Phenol		0	1: 1,000
Phenol o–mercuric chloride		64.07	1: 100,000
Ortho–nitro phenol			1: 40,000 to 1: 50,000
Meta–nitro phenol			1: 5,000
Para–nitro phenol			1: 10,000 to 1: 20,000
Mercurophen Sodium o–nitro Phenol p–mercuric hydroxide		52.8	1: 50,000 to 1: 80,000
No. 9 Sodium p–nitro phenol o–mercuric chloride		52.8	1: 50,000 to 1: 100,000
No. 15 2,2′ mercuric bis p–nitro phenol		42	1: 100,000
P–nitroso phenol			1: 1,000 to 1: 5,000

TABLE 1—*Continued*

PHENOLS

Name	Formula	Percentage of Mercury	Complete Inhibition
P–nitroso phenol O–mercuric chloride		55.9	1: 80,000 to 1: 100,000
No. 20 2,2' hydroxy 4–nitroso–mercury diphenyl		48.2	1: 100,000 to 1: 300,000
Saligenin phenol o–carbinol			1: 1,000
Phenol o–carbinol 2, 4 mercuric acetate		45.5	1: 20,000
Phenol o–carbinol 2, 4 mercuric hydroxide		74.4	1: 50,000 to 1: 60,000
O–nitro phenol P–carbinol O–mercuric acetate		46.9	1: 100,000

prevention of growth of the human tubercle bacillus as phenol and two and one half times as efficient as mercuric chloride, which inhibits in a dilution of 1:40,000. We find that the nitro-phenols are more efficient than phenol, the ortho-nitro-phenol being from 40 to 50 times as strong, the para-nitro-phenol from 10 to 20 times as strong and meta-nitro-phenol only 5 times as strong in its inhibitory action on the tubercle bacillus. The quinoidal change which is supposed to take place in the phenol molecule on the substitution of NO_2 for hydrogen probably accounts for this, since that change occurs most readily with NO_2 in the ortho position and least readily with it in the para position.

Mercurophen and Na9 are isomers and the ortho position of the mercury in the latter seems more favorable than the para position. Na15 in which mercury forms a bridge between two sodium nitro phenols is stronger in its action than either of the other compounds, although its content of mercury is considerably less. In this, also, the bridge occupies the ortho position. Although p-nitroso phenol has lower inhibitory power than p-nitro-phenol, p-nitroso-phenol o-mercuric chloride has about the same power as Na9 which has the same chemical formula except that it has NO_2 in place of NO. Number 20, in which a phenol ring is substituted for the Cl and is connected to the p-nitroso-phenol by a mercury bridge in the ortho position, has greater inhibitory power than any of the other phenol compounds so far studied. The mercury saligenin compounds inhibited at from 1 : 20,000 to 1 : 60,000, although containing 45 to 74% of mercury, while the saligenin mercury compound which had one nitro group in the ortho position completely inhibits at 1 : 100,000.

Table 2 contains the results of my inhibitory tests with a number of organic mercury compounds of aniline, nitro-anilines and methyl or ethyl anilines and nitranilines. Most of these compounds are insoluble except in dilute alcohol, which itself has considerable inhibitory power. Several of the mercury aniline and mercury methyl and ethyl anilines were combined with tartaric acid or acetic acid to form salts which are readily soluble in an alkaline solution so dilute that it does not itself check the growth of the organisms. The nitranilines, however, would not form soluble salts with any acids tested. The results as shown in table 2, seem to indicate that the addition of one or two methyl or of one or two ethyl groups does not materially affect the efficiency of these compounds as antiseptics and that the mercury is mostly, if not wholly, responsible for the fact that the mercury compounds of aniline are from 20 to 80 times as strong in their inhibitory action on the tubercle bacillus as aniline itself. On account of their insolubility, it has been necessary to use the nitro-compounds of aniline and mercury in alcoholic solutions, and even in alcohol they have not always been completely soluble down to dilutions of 1 : 1,000. At 1 : 10,000, however, they were soluble and in this dilution, the alcohol was sufficiently dilute to have very little if any antiseptic action, and growth has been completely prevented at 1 : 100,000. The nitro group in the aniline molecule has not increased the inhibitory action of this substance as much as it did that of the phenol molecule, but the increase amounts to 5 to 10 times that of aniline. In the nitranilines, the meta

TABLE 2

ANILINES

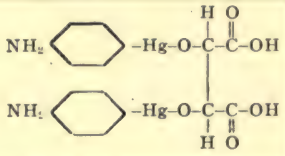
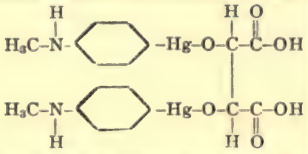
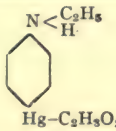
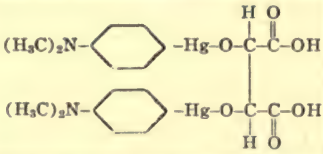
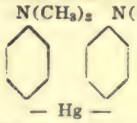
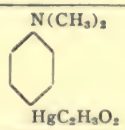
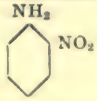
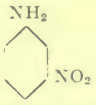
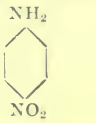
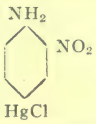
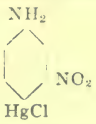
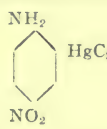
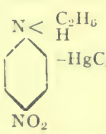
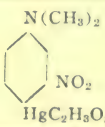
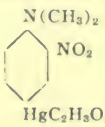
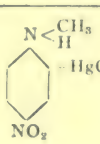
Name	Formula	Percentage of Mercury	Complete Inhibition
Aniline		0	1: 1,000
Aniline p–mercuric tartrate		53.9	1: 20,000 to 1: 40,000
Mono methyl aniline p—mercuric tartrate		50.7	1: 40,000 to 1: 80,000
Mono ethyl aniline p–mercuric acetate		52.8	1: 40.000 to 1: 60,000
40 A di methyl aniline p–mercuric tartrate		49.1	1: 20,000 to 1: 40,000
No. 37 Di methyl aniline p–mercuric acetamide		53.0	1: 40,000 to 1: 60,000
4, 4′ mercury bis di methyl aniline		45.3	1: 20,000
Di methyl aniline p–mercuric acetate		52.8	1: 20,000 to 1: 40,000
O–nitraniline		0	1: 5,000

TABLE 2—*Continued*

ANILINES

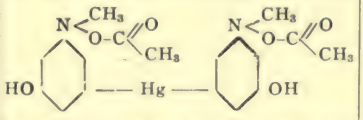
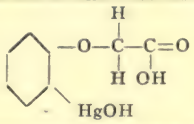
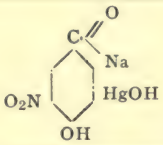
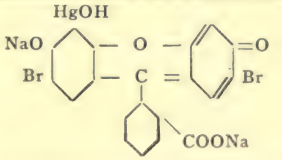
Name	Formula	Percentage of Mercury	Complete Inhibition
M–nitraniline		0	1: 10,000
P–nitraniline		0	1: 1,000 to 1: 5,000
O–nitraniline P–mercuric chloride		57.8	1: 100,000
M–nitraniline P–mercuric chloride		57.8	1: 100,000
P–nitraniline o–mercuric acetate		50.6	1: 50,000 to 1: 60,000
(14 M) Mono ethyl p–nitro aniline o–mercuric acetate		47.2	1: 100,000
No. 26 Di methyl m–nitro aniline p–mercuric acetate		47.2	1: 100,000
No. 27 Di methyl o–nitro aniline p–mercuric acetate		47.2	1: 100,000
No. 29 Mono methyl p–nitraniline o–mercuric acetate		48.8	1: 80,000 to 1: 100,000

position of the nitro group is most favorable instead of the ortho position as in the nitro-phenols. This is probably due to the fact that the quinoidal change in the ring does not easily take place in the aniline molecule.

Table 3 presents my results with several unrelated organic mercury compounds of the benzene nucleus. L. C. 27 is a very soluble mercury

TABLE 3

OTHER MERCURY COMPOUNDS OF THE BENZENE NUCLEUS

Name	Formula	Percentage of Mercury	Complete Inhibition
L. C. 27 Mercuric–bis 3–hydroxy phenyl trimethyl ammonium acetate		32	1: 1,000 to 1: 5,000
No. 55 Phenoxyl acetic acid o–mercury hydroxide		55.2	1: 10,000
No. 62 3 nitro – 4 hydroxyl 5–mercury–hydroxide benzoic acid		48.2	1: 20,000 to 1: 80,000
Mercurochrome–220		26.7	1: 10,000
Mercurochrome–205			1: 10,000
Mercurochrome–253			1: 10,000

bridge compound containing only 32% of mercury. It shows, however, very little more inhibitory action than phenol. Number 55 is soluble, contains 55% of mercury and inhibits at 10,000, while No. 62, a similar compound but having a nitro group ortho to the hydroxyl and with only 48% of mercury, inhibits at 1 : 80,000. Three modifications of the mercurialized fluorescein compound made by Young, White and Schwartz all have the same inhibitory power, 1 : 10,000.

SUMMARY

The power of phenol to inhibit the growth of the human tubercle bacillus is greatly increased by the substitution of a mercury salt in place of one of the hydrogens,—hence a mercury united by one bond to carbon.

It is also increased by the substitution of one NO_2 group for one hydrogen in the ring.

The position of the NO_2 group has much to do with the degree of increase of the inhibitory power, the ortho position being most favorable and the para position next. This is probably due to a quinoidal change in the phenol nucleus.

The position of the mercury group also has much influence on the degree of increase of inhibitory power, the ortho position seeming most favorable.

The mercury bridge compounds seem also to have a high inhibitory power, at least in the two compounds tested, in both of which the bridge occupies the ortho position.

Although saligenin or phenol carbinol has the same inhibitory power as phenol, the mercury derivatives of this have a greatly increased efficiency, varying somewhat with the percentage of mercury; one compound, however which has less mercury but in which both a nitro and a mercury group occupy the ortho position with respect to the hydroxy group, has a higher inhibitory power.

In the aniline compounds also the substitution of a mercury group greatly increases the efficiency.

The nitro group also increases the inhibitory efficiency but not in the same order of position as in the nitro-phenols, since the quinoid change does not readily take place in the aniline nucleus. However, the aniline compounds having the nitro group in the ortho position and the mercury salt in the para position seem more efficient than if the order is reversed.

Methyl and ethyl groups do not materially affect the antiseptic power of the aniline compounds, although these compounds having methyl or ethyl groups plus nitro groups plus mercury groups have a very high antiseptic power, not apparently varying much either with the percentage of mercury or with the relative position of the different groups.

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STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS

XXIII. THE CHEMICAL COMPOSITION OF CALCIFIED TUBERCULOUS LESIONS

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In reviewing the literature on the use of calcium and silica in the treatment of tuberculosis, we have been impressed with the small number of thorough analyses that have been made of calcified tuberculous lesions and the large amount of speculation that has been based upon the few analyses that exist. Many writers seem to look upon the calcification of tuberculous lesions as the process by which healing is accomplished, and deduce from this the conclusion that if calcium salts are given to the patient by mouth these will be deposited in the tuberculous lesions and terminate the process. This assumption disregards the abundant evidence that in human tuberculosis calcification takes place solely because the lesion has existed for a considerable time, as an inert, avascular mass, and therefore is already quiescent or healed, rather than that the healing results from the calcification. The entire subject of calcium metabolism in tuberculosis and its relation to calcium therapy of this disease will be discussed by us elsewhere. Here we wish to discuss merely the composition of calcific deposits found in healed or quiescent tuberculous lesions.

From such analyses as have been published it would seem that the chemical composition of the calcific deposits in tuberculosis differs not at all from those occurring in other pathological conditions. Two analyses of pulmonary concretions are quoted by Ott (1) which, translated into percentage figures, are as follows:

Ca phosphate	52	73
Ca carbonate	13	6
Mg salts	2	1
Fe_2O_3	3	
Silicate	1	1
Lipoids and fats	24	7
Sodium salts		1
Organic material and loss	5	11

These analyses were made in 1837 and 1858, and their accuracy is questionable. Even more recently, most of the analyses that have been made of calcium deposits have been carried out by the ashing process, which is far from accurate, since in the presence of phosphates and carbonates formed from the tissue during the ashing the calcium salts may be converted into one or the other according to the proportions and the temperature, as Wells (2) found by direct experiment.

Using a wet extraction process which eliminates this source of error he obtained the following figures, which are shown in comparison with some obtained with normal bone by other observers, who, however, used the ashing process:

	$Mg_3(PO_4)_2$	$CaCO_3$	$Ca_3(PO_4)_2$
Pathological calcification			
Bovine tuberculosis	0.84	12.8	85.9
Bovine tuberculosis	0.9	13.1	85.4
Bovine tuberculosis	1.2	11.7	86.4
Bovine tuberculosis (softened lymph node)	1.5	7.6	90.6
Human tuberculosis	1.2	10.1	87.8
Calcified nodule in thyroid	0.85	13.4	85.4
Thrombus, human	1.1	11.9	86.5
Normal ossification			
Human bone (Zalesky)	1.04	±12.8	83.8
Human bone (Carnot)	1.57	10.1	87.4
Human bone (Carnot)	1.75	9.2	87.8
Ox bone (Zalesky)	1.02		86.1
Ox bone (Carnot)	1.53	11.9	85.7

It will be noted that there is no significant difference in the composition of calcific deposits in human and in bovine tuberculosis, in spite of the fact that in bovine tuberculosis the calcification occurs in the form of minute granules scattered throughout the lesions even in an active stage, while in human tuberculosis the calcium is deposited in large masses in nearly or quite inactive tubercles.[1]

As indicated by these figures, the composition of the inorganic salts in calcified areas in the body seems to be practically the same, if not identical, whether the salts are laid down under normal conditions (ossi-

[1] Although many forms of calcified tissues, normal and pathological, contain microchemically demonstrable iron, Gierke (*Virchow's Arch., 1902, clxvii, 318*) found none in calcified tubercles in the lungs and peribronchial lymph nodes.

fication) or under pathological conditions; and whether in tuberculosis or some other lesion that produces avascular necrotic masses in which lime salts accumulate. This, however, is to be expected, since in each case the calcium salts must come from the blood, where they are held in true solution or in colloid suspension by the proteins, either as the carbonate and phosphate themselves, or as calcium-ion-protein compounds, or perhaps both. This suspension or solution is an unstable condition, possible only because of the extremely small proportion of calcium in the plasma (about 1:10,000), and, therefore, capable of being overthrown by increased alkalinity of the blood, changes in the proteins or CO_2 content, or changes in the quantity or composition of the calcium salts. It is probable, from the work of Barille, that the calcium of the blood exists as a soluble complex double salt, tribasic calcium-carbon-phosphate ($P_2O_8Ca_2H_2:2CO_2$, $(CO_3H)_2Ca$), this compound being possible because of an excess of CO_2. Loew (3), however, believes that the calcium is carried in the blood as bicarbonate. He found that a mixture of $NaHCO_3$, Na_2HPO_4 and $CaSO_4$, dissolved by saturation with CO_2, deposited pure $CaCO_3$ without any phosphate when the CO_2 was driven off by a current of air, but, if heated, a mixture of $CaCO_3$ and $Ca_3(PO_4)_2$ resulted.

Even were the deposits originally different in composition from the bones, this would naturally tend to become the same, since the blood continually passing between the bones and the calcified areas would bring about an equilibrium in the course of time, as pointed out elsewhere (4). Calcified tubercles, in common with other calcified lesions, may eventually become transformed into true bone, not only with bone corpuscles and Haversian systems, but also with typical functioning marrow. This morphological change, however, does not involve any appreciable change in composition, for the reasons given above. Because of the extreme avascularity of calcified tubercles, however, this transformation into bone occurs less frequently than in the case with calcific deposits that are not so completely shut off from the blood vessels.

Zickgraf (5) is authority for the statement that "lung stones," which usually are the calcified centres of tubercles set free by suppuration about them, contain much silica. He gives the following figures for the composition of four such stones:

	WEIGHT IN MGM.	PERCENTAGE OF SiO_2	PERCENTAGE OF CaO
I	110	12.45	10.54
II	100	5.70	10.4
III	225	5.02	5.98
IV	490	4.71	41.08

Gerhartz and Strigel (6), however, found reports of three analyses in the literature, in one of which no silica was found, in another a trace, in the third but 1 per cent, and their own analysis of another specimen revealed no silica.

Scherer (7) states that in a hospital population of 16,000 cases, chiefly pulmonary, there occurred 21 cases in which it could be definitely established that at some time "lung stones" had been coughed out and he describes several of his cases in detail. He reports analyses of three such specimens which he admits are not any too accurate, with the following results:

	STONE I		STONE II		STONE III	
	Totals	Percentage of inorganic	Totals	Percentage of inorganic	Totals	Percentage of inorganic
	mgm.		*mgm.*		*mgm.*	
Weight of stone	520		976		460	
Calcium carbonate	104	57.3	220	31.4	80	20.5
Calcium phosphate	112	31.5	470	67.1	295	75.1
Silicic acid	17	4.9	Traces		6	1.6
Magnesium salts	23	6.4	11	1.5	8	2.1
Fat and cholesterol	88		85		32	
Undetermined	76		190		34	

He comments on the low content of silicic acid in comparison with the figures published by Zickgraf. Most of the expectorated stones are very irregular in form and dissolve readily in strong acid. They probably generally come from calcified lymph nodes, although they may come from calcified nodules set free in the formation of cavities in the lung tissue. He has examined several specimens of decalcified lung stones with various agents but has never been able to find either tubercle bacilli or any definite structure.

Kahle (8) reports finding 1.6 gm. SiO_2 per kilo of dry weight in a calcified tuberculous hilum node of a child, but gives no figures for the other inorganic elements. He says that Weyland found in normal

bovine bronchial nodes 0.14 gm. SiO_2 per kilo dry weight, in tuberculous nodes 0.27 gm., and in calcified tuberculous nodes 1.54 gm.

In the pus of tuberculous bone abscess, silica seems to be far from abundant, for Schulz (9) found in two specimens but 0.0532 and 0.0461 per cent of SiO_2. Robin (10), indeed, says that the tuberculous lung contains less silica than the normal lung, but apparently this is based on two analyses only.

The four following analyses of lung stones are cited by Ott and von Hoesslin (11):

	I	II	III	IV
Calcium phosphate	52.0	72.8	69.9	72.8
Calcium carbonate	13.0	6.0	9.1	5.4
Magnesium phosphate		1.0		0.9
Magnesium carbonate	2.0			
Fat and cholesterol	24.0	7.0	20.1	7.2
Other organic substances	4.0	10.0		9.8

In other words, allowing for difficulties in analysis, the calcified masses set free occasionally in tuberculous lungs and peribronchial nodes, exhibit quite the same composition as other areas of pathological calcification, resembling bone ash in their proportions of calcium and magnesium phosphate and carbonate, with probably admixture at times of silicates deposited from inhaled dust, and also sometimes traces of iron from old hemorrhages.

The group of German clinicians who of late years have so enthusiastically advocated the use of silica in the treatment of tuberculosis (12) have laid much stress upon Zickgraf's finding of silica in calcified tuberculous lesions and lung stones. They base their therapeutics on the theory that silica is an important element in the formation of scar tissue, and cite these figures of Zickgraf as supporting the view that silica is concerned in the healing of tuberculous lesions. In so doing they completely disregard the fact that the lung always contains much inhaled silicious dust, as also do the peribronchial nodes, and that this dust is concentrated in scars just as we see the coal dust accumulated in the healed tuberculous lesions. Hirsch (13) found in four ordinarily pigmented lungs of adult city dwellers from 0.040 to 0.69 gram of SiO_2 per lung, which constituted from 0.04 to 0.72 per cent of the dry weight, or from 1 per cent of the total ash in the lung with the least silica up to 14.5 per cent of the ash in the lung with the most silica. These

figures show that with our ordinary inhalation of dust particles the lungs come to contain a far larger proportion of silica than Zickgraf found in his calcified tubercles, on which finding so much weight has been laid by advocates of silica as a therapeutic agent in tuberculosis. In gold mine workers in South America, McCrae (14) found from 19 to 48 per cent of the ash to be silica.

Although these figures warrant the assumption that the silica found in calcified tuberculous lesions in the lungs and peribronchial nodes by Zickgraf and others is derived chiefly if not entirely from inhaled silicious dust, we considered it advisable, in view of the use that has been made of these findings, to secure more direct evidence on the subject. This we have done by comparing the composition of calcified tuberculous lesions of the peribronchial nodes, in which inhalation silicosis is probable, with the composition of calcified tuberculous mesenteric lymph nodes, in which this source of silica can be excluded, and also a calcified tuberculous pleural exudate.

METHOD OF ANALYSIS OF CONCRETIONS

The material was taken from the 80 per cent alcohol used as a preservative (with the exception of the pleural exudate, which had been dried), cut into small pieces and extracted with ether in a Soxhlet apparatus for six hours. It was then dried at 110° for ten hours and weighed in a carbon dioxide flask.

The carbon dioxide was liberated by boiling in 5 per cent hydrochloric acid, and determined gravimetrically by difference of weight of Geissler bulbs.

The hydrochloric acid solution with the undissolved tissue was made up to 200 cc. and filtered. One aliquot portion of the filtrate was analyzed for inorganic phosphorus, and one was analyzed for oxalate.

The phosphorus was determined by Pemberton's modification of Neumann's method, which consists of the titration of the ammonium phosphomolybdate, with $\frac{N}{2}$ NaOH.

The oxalate was precipitated by excess ammonia. The precipitate was filtered, washed with 2 per cent ammonia and titrated with potassium permanganate.

The remaining mixture was transferred to a platinum dish and evaporated to dryness. The last traces of hydrochloric acid were removed by repeated additions of absolute alcohol. This removal of HCl was

necessary because nickel crucibles were used for ashing. The residue in the platinum dish was transferred with water to the nickel crucible, evaporated to dryness on the water bath and ashed.

The silica was determined in the ash by the method described by Lenher and Truog (15) with modifications needed in treating much smaller quantities of silica. The ash is treated with 30 to 60 cc. of HCl (Sp. gr. 1.07), and the mixture evaporated to dryness on the steam bath. The residue is treated with 10 to 15 cc. (depending upon the amount of ash) concentrated HCl (Sp. gr. 1.1), covered and heated on the steam bath for ten minutes. The solution is diluted and filtered immediately. This filtration removes most of the silica. The filtrate is evaporated to dryness and dehydrated for two hours at 110°. The residue is taken up with 8 cc. HCl (Sp. gr. 1.1), covered and heated on the steam bath for five minutes, diluted with 25 cc. water and filtered immediately. The two filter papers containing the silicon dioxide are placed in a platinum crucible and ignited, ashed and weighed. The silica is determined by loss on volatilizing with hydrofluoric acid, after the addition of a few drops of sulphuric acid. The filtrate from the silica was made up to volume, and aliquots taken for calcium and magnesium, and organic plus inorganic phosphorus. The calcium and magnesium were determined by the method of McCrudden (16). The results of the analyses are given in table 1.

All the materials analyzed were densely calcified masses, undoubtedly old healed tuberculous lesions, except specimen 993 which consisted of a group of caseous lymph nodes, both mediastinal and mesenteric, which showed only an early stage of calcification, with soft chalky foci in the caseous areas. This specimen was thoroughly extracted with boiling amyl alcohol, which should have dissolved out any calcium soaps that might have been present in so early a lesion, but 0.0005 gm. of calcium was thus obtained, which was not more than might have been dissolved out by such extensive amyl alcohol extraction of pure inorganic salts of calcium. After the liberation of the carbon dioxide, an aliquot part of the mixture, in solution in hydrochloric acid, was made alkaline with ammonium hydroxide. The precipitates which were formed and which might have been calcium oxalate, were washed with 2 per cent ammonium hydroxide and titrated with potassium permanganate which was reduced as it is by oxalates.

TABLE 1

Analysis of calcified tuberculous lesions

	TOTALS AND PERCENTAGES OF DRY WEIGHT						TOTALS AND PERCENTAGES OF INORGANIC MATERIAL				
	Dry weight	SiO_2	Ca	P inorganic	Mg	CO_2	Total inorganic	SiO_2	$CaCO_3$	$Ca_3(PO_4)_2$	$Mg_3(PO_4)_2$
	grams										
983. Peribronchial	1.0898	0.0027 gm. 0.24 %	0.2736 gm. 25.1 %	0.1576 gm. 14.46 %	0.0084 gm. 0.77 %	0.0429 gm. 3.9 %	0.8829 gm. 89.2 %	0.0027 gm. 0.30 %	0.0973 gm. 11.02 %	0.7525 gm. 85.2 %	0.0304 gm. 3.44 %
978. Peribronchial	0.7234	0.0094 gm. 1.2 %	0.0870 gm. 12.02 %	0.0512 gm. 7.07 %	0.0181 gm. 2.5 %	0.0112 gm. 1.5 %	0.2795 gm. 26.8 %	0.0094 gm. 3.3 %	0.0254 gm. 9.0 %	0.1790 gm. 64.9 %	0.0657 gm. 23.6 %
1013. Subpleural nodule	0.6022	0.0038 gm. 0.63 %	0.1576 gm. 26.1 %	0.0960 gm. 15.7 %	0.0007 gm. 0.11 %	0.0153 gm. 2.5 %	0.5185 gm. 86.1 %	0.0038 gm. 0.73 %	0.0347 gm. 6.79 %	0.4775 gm. 92.0 %	0.0025 gm. 0.48 %
1028. Peribronchial	3.4203	0.0119 gm. 0.35 %	0.6955 gm. 20.3 %	0.3940 gm. 11.5 %	0.0214 gm. 0.62 %	0.0694 gm. 2.0 %	2.1260 gm. 62.1 %	0.0119 gm. 0.56 %	0.1575 gm. 7.4 %	1.8780 gm. 88.3 %	0.0786 gm. 3.5 %
1004. Peribronchial	3.4027	0.0594 gm. 1.7 %	0.5984 gm. 17.5 %	0.3320 gm. 9.7 %	0.0224 gm. 0.65 %	0.0791 gm. 2.3 %	1.8847 gm. 55.4 %	0.0594 gm. 3.1 %	0.1795 gm. 9.5 %	1.5645 gm. 83.0 %	0.0813 gm. 4.3 %
1043. Peribronchial*	0.7446	0.0051 gm. 0.68 %	0.1917 gm. 25.7 %	0.1222 gm. 16.4 %	0.0587 gm. 0.68 %	0.0222 gm. 2.9 %	0.6294 gm. 84.5 %	0.0051 gm. 0.81 %	0.0503 gm. 7.9 %	0.3610 gm. 57.5 %	0.2130 gm. 33.7 %
1023. Mesenteric	1.3340	0.0002 gm.	0.2900 gm. 21.7 %	0.1526 gm. 11.4 %	0.0034 gm. 0.25 %	0.0224 gm. 1.6 %	0.8021 gm. 60.1 %	0.0002 gm.	0.0408 gm. 5.0 %	0.7490 gm. 93.3 %	0.0123 gm. 1.5 %
95–1922. Mesenteric	1.8258	0	0.4726 gm. 25.8 %	0.2516 gm. 13.7 %	0.0488 gm. 2.6 %	0.0485 gm. 2.6 %	1.3271 gm. 72.6 %	0	0.1000 gm. 7.5 %	1.0500 gm. 79.1 %	0.1771 gm. 13.4 %
993. Mesenteric plus peribronchial	4.2472	0.0022 gm. 0.04 %	0.2326 gm. 5.4 %	0.2452 gm. 5.7 %	0.0250 gm. 0.58 %	0.0530 gm. 1.2 %	0.7556 gm. 10.4 %	0.0022 gm. 0.16 %	0.1203 gm. 15.6 %	0.5424 gm. 71.9 %	0.0907 gm. 12.0 %
Dry pleural exudate	6.7525	0.0025 gm. 0.03 %	0.5079 gm. 7.5 %	0.6680 gm. 9.8 %	0.0650 gm. 0.96 %	0.2391 gm. 3.5 %	1.9411 gm. 28.5 %	0.0025 gm. 0.12 %	0.5427 gm. 27.9 %	1.1600 gm. 59.7 %	0.2359 gm. 12.1 %

* The anthracotic tissue was separated from the calcified part of the lymph node. Analysis showed 0.0049 gm. SiO_2, equivalent to 0.65 per cent in the uncalcified tissue, while the calcified material contained but 0.0002 gm. SiO_2.

Calculated as calcium oxalate, this reducing substance was found in the following amounts:

NUMBER	CALCIUM OXALATE	CALCIUM	PERCENTAGE OF TOTAL CALCIUM
	gm.	*gm.*	
1028	0.0640	0.0200	2.8
1004	0.1216	0.0380	6.3
1023	0.0710	0.0221	7.5
95–1922	0.0588	0.0183	3.8
Dry pleural exudate	0.1781	0.0557	10.9

We have no way of knowing whether this material was really calcium oxalate, although it behaved as this salt should. Probably it is not an oxalate, for such amounts of calcium oxalate have not previously been described as a constituent of calcified tissues, and a study of the calcified pleural exudate, which contained the largest proportion of this material, did not yield any recognizable oxalic acid.

It will be seen from the figures given, that in our calcified tuberculous peribronchial nodes SiO_2 constituted from 0.24 to 1.2 per cent of the dry weight, or from 0.30 to 3.3 per cent of the total inorganic material of SiO_2, or even less than Hirsch found in nontuberculous lungs. The low figures for SiO_2 undoubtedly depend on the large amount of calcium that had been deposited, as shown by specimen 1043. Here the calcified nodules were separated as carefully as possible from the rest of the lymph node and the two portions analyzed separately, which revealed that the silica was almost exclusively in the noncalcareous portion of the node. That the silica found in these tissues comes to the lungs and lymph nodes as inhaled dust is proved by the fact that of the two specimens of calcified mesenteric nodes, one contained no SiO_2 and the other at best a doubtful trace. Of the same significance is the fact that the calcified pleural exudate contained but a very minute amount of silica, probably derived by lymphatic deposition in the pulmonary pleura. In general, the figures show that calcified tuberculous lesions are composed, like other calcified tissues and normal bone, chiefly of a mixture of calcium phosphate and calcium carbonate in a proportion of approximately 10 to 1. The very small amounts of material analyzed in some of our specimens accounts for the variations from this standard, and also for the high figures for magnesium phosphate in a few cases, especially when there was much organic material present. Possibly some of the magnesium may also have been inhaled as dust.

SUMMARY

Calcified peribronchial lymph node and pulmonary lesions contain a small proportion of silica, but not more than uncalcified peribronchial lymph node or lung tissues contain in adults. This silica is derived chiefly, if not entirely, from inhaled silicious dust, for calcified tuberculous mesenteric lymph nodes do not contain appreciable amounts of silica, and only a trace was found in a calcified tuberculous pleural exudate. Therefore, these analyses furnish no chemical evidence supporting the hypothesis that silica plays an important part in the healing of tuberculous lesions. Calcified tuberculous lesions resemble in composition other calcified pathological tissues and also normal bone.

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THE LIPIN CONTENT OF ACID-FAST BACILLI

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The experiments recorded below had for their object a comparison, quantitative and qualitative, of the lipin content of acid-fast bacilli, in relation to the two factors of virulence and glycerophilism.

Since the analyses by Hammerschlag (1) in 1888 it has been well known that tubercle bacilli were, as compared with other bacteria, exceptionally rich in substances soluble in the usual fat solvents, such as chloroform, ether, petroleum ether, benzene, toluene and xylene. The percentage of material extractable has varied with different observers and methods and variety of tubercle bacillus used, and has ranged between 20 per cent and 40 per cent of the dry weight.

It was but a short step to correlate this unique high lipin content with the equally unique staining property of the bacillus, acid-fastness. In fact, even before Hammerschlag's quantitative research on the lipins present, the impression was prevalent that acid-fastness was to be associated with the fatty or waxy nature of the microörganism. Indeed, Bienstock (2) and Gottstein (3) went so far as to claim on the basis of experiment that non-acid-fast bacteria, such as B. pyocyaneus and B. typhosus, could be made acid-fast by growth on fat or wax-rich media or even by trituration in a fatty substrate. While, since their time, acid-fastness has been attributed by different investigators to various constituents of the bacillus, the majority have definitely correlated it with the fatty content, the greater number of them in turn ascribing the staining property to a fairly specific acid-fastness of tubercle bacillus wax, demonstrable in the isolated substance.

An implication naturally carried with this theory is that the other acid-fast bacteria will owe their resistance to acid-decolorization to the same property, and indeed it has been generally assumed that such bacteria as the smegma bacillus, the grass bacilli, and the so called tubercle bacilli of cold blooded animals, are, like the tubercle bacilli, rich in fatty substance. There are however few actual analyses on record to bear this

[1] Aided by a grant from the National Tuberculosis Association.

supposition out. Gurd and Denis (4), working on the acid-fast bacillus isolated from leprosy nodules by Duval, found 36.4 per cent of the dry weight of the bacillus to be extractable with chloroform. Recently Pfannenstiel (5) (who gives a comprehensive bibliography) has published analyses comparing the lipin content of tubercle bacilli of human and avian types, frog bacilli, turtle bacilli, timothy grass bacilli, and butter bacilli, which he maintained had been raised in virulence by guinea pig passage. The total lipin removed in Pfannenstiel's extractions was low, the highest recorded value being 16.47 per cent of the dry weight in the case of a butter bacillus. The lowness of their figure is to be attributed to the method used, extraction of the dried material, which had been grown on glycerol broth, with a mixture of ether and acetone. As is well known, some of the waxy material of the tubercle bacillus is difficultly soluble in ether; and the phosphatids, which, as Tamura (6) has shown, make up a considerable proportion of tubercle bacillus lipin and some of the wax, are insoluble in acetone. However, Pfannenstiel stresses the point that the figure obtained varies with the method, and, as in his experiments the same method was used throughout, the results are of value for comparison of the different bacteria examined.

His results indicated a wide variability in lipin content throughout the group, a value as low as 2.85 per cent of the dry weight being found for a tubercle bacillus of the human type. It was thought that the lipins were distributed in the cell protoplasm in varying fashion and that accordingly ease of extraction varied. The figures do not indicate any constant quantitative difference in the lipin content of virulent and avirulent strains. Qualitative examination of the extracted material was not made.

A very significant finding has recently been reported by Frouin (7). This investigator compared the lipin content of tubercle bacilli grown upon glycerol-asparagine broth and on the same medium in which the glycerol was replaced by 15 per cent dextrose plus mannitol. On the sugar media the bacilli grew with much less luxuriance. They were also 60 to 70 per cent lower in lipin content. Bovine bacilli contained from 23 to 45 per cent lipin when grown on the glycerol medium, and only 8.5 to 13 per cent on the sugar medium. Similar results were obtained for human and avian type bacilli. Unfortunately Frouin did not report qualitative examination of the lipin removed.

It has long been known that a considerable proportion of the lipin of the tubercle bacillus was not true fat, but a wax, that is to say, an ester

of a fatty acid and an alcohol of high molecular weight. The nature of this compound has been fairly well worked out by Kresling (8), Bulloch and McLeod (9), Tamura (6), and Goris (10). From Kresling's figures it may be calculated that 39 per cent of the total lipin is in the form of a nonsaponifiable alcohol. The saponification number has been variously reported around 120 to 140. As Goris points out, fats ordinarily have a saponification number of 175 to 250, while waxes have a much lower one, but not below 79 (Carnauba wax). The tubercle bacillus lipin, being a mixture of the two, would be expected to give a figure in between.

With considerable uniformity those who have isolated the wax have reported it to be acid-fast and have been inclined to attribute acid-fastness of bacilli to its presence. It is strange that analyses have not been made heretofore to determine if the same or a similar wax makes up a large proportion of the dry weight of the other acid-fast bacilli. The analyses reported in this paper deal with this problem. The method used was as follows:

Bacilli were grown upon glycerol broth containing 1 per cent peptone and 5 per cent glycerol, or in the case of certain bacilli which do not float well, namely, avian, leprosy, frog and turtle bacilli, upon the same medium made solid by the addition of 2 per cent agar. The bacilli secured were sedimented and washed in water, treated with 95 per cent alcohol, boiled twice in absolute alcohol, and finally leached thoroughly with medium-boiling petroleum ether. The alcoholic extracts were also evaporated to dryness and extracted with petroleum ether. The petroleum ether extracts were added and evaporated to dryness and the dry extract was weighed. The weight of this extract was calculated in percentage of the total dry weight, which was calculated as follows: weight of dry bacilli left after petroleum ether extraction, plus weight of alcohol-soluble residue left after petroleum ether extraction, plus weight of lipin soluble in petroleum ether. The final figure in effect represents the proportion of the alcohol-dehydrated bacillus which is soluble in petroleum ether. The saponification number was obtained by heating a known weight of the extracted lipin to gentle ebullition in a known amount of 25 per cent alcoholic potash for five hours, and titrating back the unused alkali in the usual way. The titrated material was again made strongly alkaline and evaporated to dryness. The residue was extracted with petroleum ether, and this extract was evaporated to dryness. The residue from this was extracted with chloroform and after evaporation of the chloroform, the wax, thus thoroughly separated from the soaps, was weighed.

Table 1 gives the results of analysis. With two exceptions they indicate a very high lipin content for acid-fast as compared with non-acid-fast bacilli. A staphylococcus and a hay bacillus analyzed by the same method showed only 2.8 per cent and 4.5 per cent of the dry weight as lipin. In the case of two exceptions, the avian bacillus and the leprosy bacillus, it was noted that a large amount of lipoid material, perhaps 20 per cent of the dry weight, which was soluble in warm alcohol, but insoluble in petroleum ether, was present. This substance requires further investigation. The other bacilli ranged from 20 to nearly 40 per cent lipin.

TABLE 1

Lipin content of acid-fast bacilli

	TOTAL LIPIN IN PER CENT OF DRY WEIGHT	SAPONIFICATION NUMBER	PER CENT OF TOTAL LIPINS AS NONSAPONIFIABLE LIPOID
H 37, human type tubercle bacillus	22.7	131	77.1
B 1, bovine type tubercle bacillus	22.3	139	60.0
A 1, avian type tubercle bacillus	11.0	194	35.7
Leprosy bacillus, Duval	9.7	188	27.2
Turtle bacillus	36.3	191	28.3
Frog bacillus	37.1	195	33.7
Smegma bacillus	35.6	128	4.6
Dung bacillus	34.7	125	5.4
Grass bacillus	23.4	147	9.4
Grass III bacillus	30.3	109	4.5
Timothy bacillus	20.2		
Lipin content of other microörganisms			
B. subtilis	4.4		
Staphylococcus albus	2.8		

A more significant finding however is that of the high wax content of the virulent forms. At one end of the scale we have microörganisms of scarcely questioned saprophytism, the smegma and grass bacilli, ranging in wax content (nonsaponifiable lipin) from 4.5 per cent to 9.4 per cent. At the other end are the tubercle bacilli of human and bovine type with wax values of 77 per cent and 60 per cent of the total lipin. In between are microörganisms of debated pathogenicity, avian, leprosy, frog and turtle bacilli, with values for wax of 27.2 per cent to 35.7 per cent. The saponification numbers agree with the wax findings except in the case of the smegma and grass bacilli, in which they are unexplainably low. Further research is necessary on this point. It is to be believed

that the low wax content of these bacilli means a correspondingly high content of neutral fat, but the saponification number does not bear this supposition out. An excellent agreement with theory as respects saponification number and wax content is obtained in the case of the tubercle bacilli and the avian, leprosy, frog and turtle bacilli. We have every reason for believing that the greater proportion of the extracted lipin in the case of the four last-named types was true fat.

We would not lay too much stress upon our findings with respect to virulence, however. The virulence of most of the particular strains used has not been tested recently in our laboratory. It is known that H 37 and B 1 are highly virulent, but no attempt has been made to study virulence in the others, in any animals. However, the nutritional requirements of this group have been investigated by one of us (11) and are well understood. As is generally known, glycerol is an important element in the nutrition of the tubercle bacillus. The bacteria used in this investigation decrease in their glycerophilism approximately in the order in which they are listed in table 1. It is our belief, on the basis of our results and Frouin's, that the glycerol requirement and the wax content are to be directly correlated, glycerol being a wax, but not necessarily a fat-progenitor.

SUMMARY

The bacilli of the acid-fast group are rich in lipin as compared with non-acid-fast bacilli. In the majority of cases 20 to 35 per cent of the dry weight can be readily extracted with petroleum ether after dehydration of the bacilli with hot alcohol. Tubercle bacilli of human, bovine and avian types, leprosy, frog, turtle, smegma, dung and grass bacilli, and B. subtilis and a strain of Staphylococcus albus, were examined in this investigation.

Qualitatively, there is a great difference in the lipin extracts. The bacilli studied fall into three groups as respects the proportion of the total lipin which is present as wax (nonsaponifiable lipin). This wax, as has been repeatedly shown, consists of a difficultly hydrolyzable combination of fatty acid with an alcohol of high molecular weight. The lipin of human and bovine type tubercle bacilli is 60 to 77 per cent wax. That of avian type tubercle bacilli, the so called leprosy bacilli, and the so called tubercle bacilli of frogs and turtles, ranges between 27 and 36 per cent wax, the higher value found being for the avian tubercle bacillus. The wax content of smegma, dung and grass bacilli of various types ranges from 4 to 10 per cent of the total lipin.

A closely similar grouping of the same series of microörganisms has been made before on the basis of nutrition. A similar grouping as respects virulence is of course obvious, but we do not care to stress this without further investigation of the disease-producing power of the so called tubercle bacilli of cold blooded animals. Sufficient data have, however, been secured to show that the microörganisms listed build wax somewhat in proportion to their glycerophilism, and therefore glycerol may be looked upon as a wax progenitor.

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LIPIN-PROTEIN IN RELATION TO THE ACID-FASTNESS OF BACTERIA

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The term lipin-protein is used in this paper to indicate an intimate union of fatty substances and protein, without commission to the idea that the union is necessarily a chemical one. The term is meant to cover those protein-lipin combinations or mixtures from which the lipin may be extracted only with difficulty. It is well known that such combinations are present in most living cells and modify their permeability. The union may be a true chemical one, analogous to that occurring in nucleoproteins or glycoproteins, or may be merely a fine emulsion of the lipin in a protein substrate, the individual droplets being enclosed each within a protein film.

It has seemed reasonable to the author to suppose that such combinations exist in the tubercle bacillus, as in other cells, and furthermore that they may play a rôle in its resistance to acid-decolorization after staining. It has seemed also that development of this theory may serve to harmonize some of the conflicting ideas regarding the cause of acid-fastness of tubercle bacilli and other bacteria of the acid-fast group. Without such a hope one would hesitate to add to the already vast and cumbersome literature on this subject.

Of the several chief explanations of acid-fastness there are two in much better standing than the rest. These are the one developed originally by Ehrlich, which has as its basis the relative impermeability of the tubercle bacillus to stains, and a second, representing the combined labors of many men, in which the acid-fastness of the whole bacillus is credited to the specific acid-fastness of a constituent, the peculiar lipin in which the microörganism is rich. Ehrlich (1), impressed by the difficulty of staining the bacillus, which presumably delayed its discovery, and by Koch's use of an alkali to effect staining penetration, and finding furthermore that the bacillus once stained could not be decolorized by

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the use of 33 per cent nitric acid or alcohol, as other bacteria could be, advanced the theory that the tubercle bacillus was provided with an envelope of low degree of permeability. No explanation was given as to the nature of this envelope.

With the finding later of an exceptionally high fat or wax content of the bacillus, the idea of a capsule, waxy in its nature, met with rather general acceptance, and ingenious reconstructions of the tubercle bacillus have been made on its basis and on experimental data secured (*cf.* Bergel (2)). However, a large number of investigators have definitely attributed acid-fastness to the lipin itself. Koch (3) recorded that he had learned with Proskauer that there were substances present in the tubercle bacillus, related to the fats, which were both acid and alcohol-fast and were to be considered as carriers of the peculiar staining property. Klebs (4) had isolated 22 per cent of the dry weight of the bacillus in the form of an acid-fast waxy substance, which he believed accounted for the stain-retaining power of the organism. Aronson (5), Bulloch and McLeod (6), Camus and Pagniez (7), Tamura (8) and Goris and Liot (9) made notable contributions on this subject. As a result of this combined work and that of Kresling (10), Dorset (11), Fontes (12), Panzer (13), Kozniewski (14), Bürger (15) and others, the belief has become prevalent that the acid-fastness of the bacillus is determined by its content of an acid-fast ester or esters of a solid alcohol of high molecular weight.

There are two obstacles in the way of complete acceptance of the theory that acid-fastness of the bacterium is due to acid-fastness of this single substance. These are:

1. Bacilli which have been dried and treated with fat solvents, until 20 to 35 per cent of their dry weight has been removed, remain acid-fast. This is the usual observation (Hammerschlag (16), Ruppel (17), Auclair and Paris (18), Pfannenstiel (19)), although a few men have reported the reverse.

2. Bacilli from which no fat or lipin has been removed can be rendered non-acid-fast by mechanical disintegration. The powder resulting on grinding them in a mortar, or merely between glass slides, is not acid-fast (Koch (3), Benians (20), Sherman (21)).

It is difficult to see how in the case of the second of these arguments anything which could be defined as a chemical change can have taken place. Certainly no lipin was removed. Furthermore, chemical means of destroying acid-fastness, which do not involve the use of fat solvents,

have been found. Bontemps (22) used organic acids, Salimbeni (23) trichlorhydrin, and Grimme (24) weak hydrochloric acid. A significant finding of Aronson (5) and Bulloch and McLeod (6) was that only by addition of hydrochloric acid to their lipin solvents was it possible to render the bacillus non-acid-fast. Paradoxically, these investigators used almost the same extractive for removing acid-fastness (methyl alcohol with 1 per cent hydrochloric acid) as is commonly used to demonstrate it. The explanation lies in time and temperature elements. The action of the acid was prolonged and the temperature was that of boiling alcohol. The rôle played by the acid was not definitely assigned. The suggestion is, however, carried that it aided in the extraction of the lipin, which these investigators, like others, found to be acid fast.

I have in a sense separated the procedures of Aronson and of Bulloch and McLeod in the following way: Acid-fast bacilli were dehydrated with cold alcohol, treated with hot absolute alcohol and then leached with petroleum ether until no more fatty substance was extractable. The dry residue was then treated with normal hydrochloric acid (3.6 per cent) for 48 hours at 37°C., washed in water, dehydrated with alcohol and again leached with petroleum ether. Examinations for acid-fastness were made before and after the acid treatment. Eight types of acid-fast bacilli and one of non-acid-fast bacillus were examined. The results are summarized in table 1.

They indicate that treatment with acid affects previously thoroughly extracted bacilli in such a manner that renewed extraction with petroleum ether removes more lipin. This is as noticeable in the case of *B. subtilis* as in that of the acid-fast bacilli. A finding of significance is that before the acid treatment the bacillary form is preserved, while in the course of the acid treatment it is lost. The conclusion seems warranted that intactness of the bacillary form is in some way associated with the manner of deposition of the lipin within the cell protoplasm, and that the acid profoundly modifies that manner of distribution. This may be either by actual hydrolysis of a chemical lipin-protein combination, a very common type of acid effect, or by hydration of the protein substrate in which the lipin is emulsified, with an accompanying decrease in the dispersion of the lipin, the type of reaction which has been emphasized by M. H. Fischer (25) in his researches on fatty degeneration. It is believed that according to either hypothesis lipin would be rendered more readily extractable.

A qualitative examination of the lipin extracted after the acid treatment has been made limited because only a small amount of material was available. It appears to be the same in the case of all the bacilli examined, including the non-acid-fast *B. subtilis*. It is a colorless, hard, brittle, glassy substance, looking almost like a thickening of the bottom of the container when solidified in a glass beaker. It is soluble in ether, chloroform, petroleum ether and benzene, slightly soluble in hot alcohol,

TABLE 1

BACILLUS	LIPIN REMOVED BY PETROLEUM ETHER BEFORE ACID TREATMENT, IN PERCENTAGE OF DRY WEIGHT OF BACILLUS	ACID-FASTNESS OF DEFATTED MATERIAL BEFORE ACID TREATMENT	PRESERVATION OF BACILLARY FORM OF DEFATTED BACILLI	ACID FASTNESS OF DEFATTED BACILLI AFTER THOROUGH GRINDING BETWEEN GLASS SLIDES	ADDITIONAL LIPIN REMOVED BY PETROLEUM ETHER AFTER ACID TREATMENT, IN PERCENTAGE OF DRY WEIGHT OF BACILLUS	LIPIN REMOVABLE AFTER ACID TREAMENT IN PERCENTAGE OF TOTAL LIPIN	ACID-FASTNESS AFTER ACID TREATMENT	PRESERVATION OF BACILLARY FORM AFTER ACID TREATMENT
H 37, human type tubercle bacillus	22.7	+	+	0	7.8	25.6	0	0
B 1, bovine type tubercle bacillus	22.3	+	+	0	3.8	14.5	0	0
A 1, avian type tubercle bacillus	11.0	+	+	0	0.8	6.8	0	0
Leprosy bacillus, Duval	9.7	+	+	0	4.1	29.7	0	0
Turtle bacillus	36.3	+	+	0	4.8	11.6	0	0
Frog bacillus	37.1	+	+	0	4.1	10.0	0	0
Smegma bacillus	35.6	+	+	0	2.3	6.1	0	0
Grass bacillus	23.4	+	+	0	2.3	9.0	0	0
B. subtilis	4.4	0	+	0	5.1	53.6	0	Spores left intact

insoluble in cold alcohol, and insoluble in acetone. It melts, but not sharply, between 42° and 48°C. It is acid-fast. Further chemical examination has not yet been made. The melting point, solubility reactions and physical characteristics, however, suggest that it is the same substance as that which is known to make up a large proportion of the readily extractable wax of the tubercle bacillus, which has been considered mykol laurate by Goris. According to this conception the readily extractable wax is simply in the nature of a stored wax in addition to,

and less essential to, the vitality of the cell than the firmly bound portion. There is reason for believing that some of it is definitely extracellular, forming part of the matrix in which tubercle bacilli lie. As pointed out by Long and Campbell (26), wax makes up a much larger proportion of the total lipin in tubercle bacilli than in saprophytic acid-fast bacilli. The matrix in which true tubercle bacilli lie is, in my experience, denser than that surrounding the saprophytes. This matrix, somewhat acid-fast before petroleum ether treatment, loses its resistance to decolorization after petroleum ether extraction. It probably contains acid-fast wax.

DISCUSSION

It is thought that in the demonstration, recorded above, of association between integrity of bacillary cell and its content of lipin-protein some harmony is achieved in the theory of acid-fastness. The conception seems warranted that acid-fastness is the result of two factors, first, the presence of an acid-fast wax, and, second, and more important, a manner of disposition of a portion of that wax in the cell protoplasm, such that the action of destaining agents, once the organism is stained, is impeded. The second factor is considered probably the more important, since it has been repeatedly and conclusively demonstrated that mere mechanical disintegration of the cell, as well as the disruptive action of certain nonlipolytic chemical reagents, entirely destroys acid-fastness. It must be conceived that the manner of disposition of the wax in the cell wall varies with different bacteria, for it has been shown that at least one bacterium which is not acid-fast, *B. subtilis*, contains similar lipin-protein or firmly bound lipin.

A question which obviously arises is this: If the wax itself is acid-fast, how is it that mechanical disintegration of the cell, which removes no wax, removes acid fastness? This is to be explained on the basis of extremely fine emulsification and the fat, shown by Tamura, that the staining of tubercle bacillus wax by carbol-fuchsin is due to physical, not chemical, union. It is well known that fat highly emulsified in protein will not take fat-soluble stains. The conception of fine emulsification has been used by M. H. Fischer to explain the masked condition of the normal cell lipin. Fischer and the author have found, furthermore, in the former's laboratory, that the color of a mixture of fuchsin-stained wax in soap or protein solution can be almost discharged by increasing the degree of dispersion of the stained wax in the colloidal substrate.

A point of practical importance, which comes out of the theory of acid-fastness here developed, is this: Although a notion commonly held in the therapeutics of tuberculosis, namely, that loss of acid-fastness of the infective agent means the successful action of a destructive agent which has penetrated the waxy barrier, may be correct, there is a lack of precision in the idea that such penetration and disruption of the cell body means lipolysis. It is more reasonable to suppose that what has been accomplished is not a splitting or solution of the wax itself, but a separation of it from its protein combination. Lipases and esterases are not concerned in this action, and, if it is accomplished, the action of lipases and esterases may well be dispensed with. In fact, it is somewhat straining to the credulity of one who has boiled tubercle bacillus wax five hours with 25 per cent potassium hydroxide without splitting it, to imagine weak enzymes in aqueous solution attacking it so vigorously and successfully, even granting that the hydrolysis of certain compounds such as proteins, requiring concentrated chemicals *in vitro*, takes place easily in the body.

It is interesting to note that Schlossberger (27) has recently suggested that a situation such as that described in this article may exist in the tubercle bacillus. This author, who argues that the greater susceptibility of the virulent acid-fast bacilli to aniline dye disinfectants does not agree with the hypothesis that the virulent forms are provided with a stronger wax capsule, adds, "On the other hand it is in no way excluded that perhaps the fat distributed in the inner parts of the protoplasm and therefore not extractable, or extractable with difficulty, partakes in some way in the characteristic staining property."

SUMMARY OF EXPERIMENTAL RESULTS

Bacilli of the acid-fast group which have been defatted as thoroughly as possible with alcohol and petroleum ether, and from which 20 to 35 per cent of the dry weight has been removed in material soluble in petroleum ether, remain morphologically intact and acid-fast.

Such bacilli still contain from 1 per cent to 8 per cent of the dry weight in the form of lipin firmly bound to protein in a union, chemical or physical, of such sort that extraction with fat solvents is impossible. The great variance in the amount present, calculated in percentage of the total dry weight, suggests that the union is not a chemical one. This lipin may be released and rendered extractable in petroleum ether by 48 hours' treatment of the once defatted bacilli with normal hydrochloric

acid. Simultaneously, the integrity of the bacterial cell and its acid-fastness disappear. The lipin removable after the acid treatment appears to be the same in the case of all microörganisms examined, namely, tubercle bacilli of the human, bovine, and avian types, leprosy bacilli and frog, turtle, grass and smegma bacilli, and *B. subtilis*. It has the same characteristics as the wax more easily isolated fom the tubercle bacillus and is probably a mixture containing the mykol of Tamura and mykol laurate. *B. subtilis*, which is non-acid-fast, and which has a low total lipin content, contains the same firmly bound lipin in the same amount as is found on the average in acid-fast bacilli, about 5 per cent of the dry weight. However, while this firmly bound lipin usually makes up considerably less than 30 per cent of the total lipin in acid-fast bacilli, it makes up over 50 per cent in the case of *B. subtilis*.

Association of cell integrity, content of lipin-protein, and acid-fastness is demonstrated. It is thought that the disposition of the lipin-protein plays a rôle in acid fastness. The finding of similar lipin-protein in *B. subtilis*, which is non-acid-fast, requires the conception of a different manner of disposition in the two types of microörganism.

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PRIMARY SPONTANEOUS TUMORS IN THE KIDNEY AND ADRENAL OF MICE

STUDIES ON THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

SEVENTEENTH COMMUNICATION

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Primary tumors of the kidney occur not infrequently throughout the animal kingdom, and, in general, seem to exhibit the variations and peculiarities seen in human renal tumors. As an indication of the comparative pathology of renal tumors the following review of the literature is presented:

Mouse: Few cases of primary renal tumors have been described among the great numbers of other tumors found in this species. Tyzzer (1) found 4 renal growths, which he interprets as hypernephromas, among 83 primary tumors in mice. One of these mice, an old female, had also an adenoma of the lung, a carcinoma of the ovary, and a lymphoma infiltrating both kidneys; another had an adenoma in the lung. At the time this paper was written the interpretation of hypernephroma was more liberal than at present, and the illustrations might now be interpreted by many pathologists as papillary adenoma of the kidney, which was Tyzzer's original diagnosis in two of his cases.

Among the 300 mice with spontaneous tumors described by Haaland (2), there were but two renal tumors. One was a large growth in the kidney of a twenty-two months old male mouse, and had invaded a vein but produced no metastases; microscopically the structure was that of an adenocarcinoma. The second tumor, in a mouse of the same sex and age, resembled

perfectly in structure a human hypernephroma; there were no metastases, although the lung contained an adenoma. Inoculation of the hypernephroma into 40 mice was without result.

Murray (3) also described a spindle-cell growth surrounding the kidney without infiltrating it, apparently a primary retroperitoneal sarcoma and related to the kidney by position only.

Further than the above we can find no reports of primary renal tumors in mice, although Stumpf (4) has contributed a discussion of the behavior of carcinomas inoculated into the kidney.

Rats: Of 103 tumors found in 100,000 rats autopsied in plague work by McCoy (5), 11 were in the kidney, and were classified as 4 adenomas, 6 carcinomas, and 1 papilloma. Woolley and Wherry (6) in 23,000 rats found 22 tumors, of which 3 were in the kidney; all were of renal-cell type. Bullock and Rohdenburg (7), in a compilation of the literature on rat tumors, found 123 (including those cited above) of which 7 were fibroadenomas, 1 a papilloma, and 8 carcinomas of the kidney. Among 32 cases of their own in laboratory white rats, there were 10 adenomas of the kidney. Therefore, of 155 rat tumors 26 were of renal origin. A further case of carcinoma of the kidney in a wild rat was reported by Beatti (8). Loewenstein (9) described tumor-like papillary outgrowths arising in the pelvis of the kidney, as well as in the ureter and bladder, of rats infected with Trichodes crassicauda specifica.

Since sarcomas are much more common than carcinomas in rats, the fact that all the renal tumors in rats are of epithelial structure is of interest.

Squirrels: In 250,000 ground squirrels (Citellus beecheyi) autopsied in plague work, McCoy (10) found 8 tumors, of which one was described as an angiosarcoma of the kidney. A case of hypernephroma in the kidney of a grey squirrel (Sciurus carolinensis pennsylvanicus) was reported by Fox (11).

Birds: Fowls are rarely subject to renal tumors. Of 880 examined by Curtis (12), 79 had tumors, 5 of which were in the kidneys; but these were not microscopically corroborated and there is reason to doubt their true neoplastic nature. In 34 cases of tumor in fowls compiled by Wernicke (13), not one was

primary in the kidney although in 3 cases renal metastases were found. In 852 autopsied fowls Bürger (14) found 12 tumors of which none was in the kidney, although a sarcoma of the ovary had produced metastases in the kidney in one case.

The review of the literature on tumors in fowls and birds by Joest and Ernesti (15) who collected 112 cases and added about 50 more, reports no further renal tumors.

Other birds seem to have renal tumors perhaps more frequently, as is indicated by the following reports: White (16) described a fibrosarcoma in the kidney of a goose. Fox (17) reported in the kidney of a male chestnut eared finch (Amadina castanotis) a medullary carcinoma, becoming scirrhous in places, with metastases to the lungs, and three cases of benign adenoma of the kidneys in undulated grass parrakeets (Melopsittacus undulatus). He had previously reported (18) two cases of papillary cystadenoma and also a "cyst adeno-carcinoma papilliferum"(19) in the same species; besides an adenocarcinoma of the kidney in a saffron finch (Sycalis flaveola), (20) and a spindle-cell sarcoma of the left kidney with metastasis into the left tibia in a scaly ground dove (Scardapella squamosa) (21). Seligmann (22) reported as a diffuse carcinoma a growth which involved both kidneys, with metastases in the liver and mesentery, in a Chilian pintail (Dafila spinicauda) which was twenty-six years old. Baird (23) reported a case of keratinizing epithelioma in the kidney of a fowl, and Borrel and Masson (24) also described a renal tumor in a fowl which showed both cylindrical and squamous elements.

Domesticated mammals. Rabbits, which are less subject to tumor than most mammals, seem to have a relatively large proportion in the kidney, for Scott (25) stated that of 39 new growths reported, 5 were benign renal adenomas, resembling Wilms' tumors in structure.

Swine also seem to be particularly subject to renal tumors, for of the 12 cases of tumors in swine collected by Sticker, 7 arose in the kidney, and other cases reported since then indicate the same tendency. Many of these tumors were of the mixed embryonal tumor type.

In horses renal tumors are far from rare. In Sticker's (26) compilation, of 509 cases of malignant tumors in horses 37 were in the kidneys. McFadyean (27), in 63 cases of tumor in animals included 5 in the horse kidney, none with metastases. Of 142 equine neoplasms among 77,224 horses slaughtered in Japan, there were 9 in the kidney, as compared with 49 in the testicle (Kimura) (28).

According to Trotter's (29) figures, renal tumors are much less frequent in cattle, for of 305 cases but 1, a colloid cancer, arose in the kidney; but Sticker's figures show 10 of 78 bovine tumors in the kidneys. Steinke (30) described as hypernephroma a tumor of the kidney in a cow. Roussy and Wolf (31) in their review on cancer in animals, gave a picture of a tumor from a bovine kidney resembling a hypernephroma, and stated that cancer of the kidney in horses resembles that in man, while in swine the adenosarcoma of the kidney is among the most common tumors. Cadiot (32) quoted a case of an enormous cancer of the kidney in a mare.

No reports of cases of renal tumors in sheep can be found.

Of 766 primary cancers in dogs in Sticker's tables 19 were in the kidney. McFadyean also described two renal tumors in dogs, each case exhibiting metastasis. No renal tumors appeared in the 21 cats with tumors in Sticker's lists, nor in Roffo's 7 cases (33) nor in Murray's 11 cases (34) but Teutschlaender mentioned the cat (Kater) on his list of animals in which carcinoma of the kidney has been reported. Murray also described 48 cases of tumors in dogs, 12 in horses, and 18 in cows, with none arising in the kidneys.

We are indebted to Dr. L. E. Day for his summary of 316 tumors found among 2000 animal specimens sent to the Chicago Laboratory of the Bureau of Animal Industry. These do not represent all the tumors that are observed in the slaughter house, but merely specimens sent to the laboratory by the inspectors when in doubt concerning the diagnosis. Among 175 tumors of cattle there were no renal tumors although there were four adrenal tumors, two diagnosed as sarcomas and two as hypernephromas. Among 93 tumors in pigs no less than 52 were in the kidney,

47 being diagnosed as embryonal adenosarcomas and 5 as sarcomas; there were no adrenal tumors. In considering the tumors in swine it is to remembered that nearly all swine are slaughtered before they are two years old, so that not many of the slaughtered animals have reached an age for developing carcinomas. Among 48 tumors from sheep none whatever were found in the kidney or adrenal. These figures emphasize the infrequency of renal tumors in cattle and sheep and the frequency of mixed tumors of the kidney in swine, which resemble the typical mixed tumors of the human kidney.

Other incidental cases are: Sarcoma of the kidney in a swine (Hamburger) (35), the report of which leaves some uncertainty whether the growth may not have been leukemic.

Papillary adenoma in the left kidney in a mongoos lemur (White) (36). A tumor involving the kidneys, liver, lungs, and ovaries of a python, the primary site not being determined (Bland-Sutton) (37). A leiomyoma in the kidney of a horse, described by Wells (38). Pick (39) described a soft adenoma involving both kidneys of an eel. Williams (40) stated "A malignant renal tumor (derived from an adrenal 'rest') has been described by Bland-Sutton in a marmot." In Teutschlaender's compilation (44) were listed the following cases from the literature: Hemangioma of the kidney in a horse; carcinoma of the kidney in fowl, frog, pike, and buzzard.

While the above compilation is certainly not entirely complete, it serves to bring together much of the literature on the comparative pathology of renal tumors, and to indicate their general distribution and frequency.

Adrenal tumors. We can find no reports of primary adrenal tumors in mice, despite the great numbers of mice that have been examined at autopsy and the not infrequent occurrence of adrenal tumors in other species. Thus, Kimura (42) found in the literature records of adrenal tumors in 24 horses and 46 cattle, and he himself found 5 adrenal tumors among 46 tumors from horses. The extensive discussion of the comparative pathology of adrenal tumors by Steinke (43) mentioned tumors only in horses and cows, commenting on the absence of such tumors in

other species except for an adrenal tumor of parasympathetic-cell character in a two-year-old swine, reported by Klawitter.

There is, however, the interesting case reported by Smallwood (44) as a carcinoma of the kidney of a frog and believed to be derived from the adrenal tissues. Murray, who reëxamined this specimen, corroborated its carcinomatous nature, but his statement suggests that he was not convinced that it developed from adrenal rather than renal cells. The illustrations in Smallwood's article suggest a papillary renal tumor.

Fox (45) described as a hypernephroma a tumor that arose in the adrenal of a California hair seal (Zalophus californianus), and also described a hypernephroma in the adrenal of a brown cebus (Cebus fatuellus) (46).

As stated previously, in the 316 animal tumors examined by Day in the Chicago Stock Yards, but 4 arose in the adrenal, all in cattle, 2 being diagnosed as sarcoma and 2 as adenoma.

RENAL TUMORS IN MICE IN THE SLYE STOCK

In 33,000 autopsies performed on mice of the Slye stock, but 16 cases of unquestionable primary tumor arising in the kidney have been observed, supporting the impression that the kidney of the mouse is not among the common sites of primary neoplasm. These renal tumors are, according to the histological evidence, to be classified as follows: 1 carcinoma, 3 adenomas, 1 hypernephroma, 7 sarcomas, 3 mesotheliomas, and 1 sarcoma in the renal pelvis. It will be noted that we have observed no instances of the mixed tumors of the kidney that are so common in man and some other species, nor have we had any papillary tumors of the renal pelvis. The features presented by these several types of renal tumor are best described by giving briefly the findings in each of the several cases that we have observed.

EPITHELIAL TUMORS OF THE KIDNEY

1. Primary carcinoma of the kidney (fig. 1). Female mouse (1934). This mouse showed a white solid mass in the right kidney about the size of a normal kidney. Beyond this there were no

abnormalities found, except that the spleen was about three times the normal size. The left kidney was normal, and no metastases were found. Microscopically the tumor is composed of cells of epithelial type, but without the characteristics of either adrenal cells or of tubular epithelium of the kidney. They are arranged

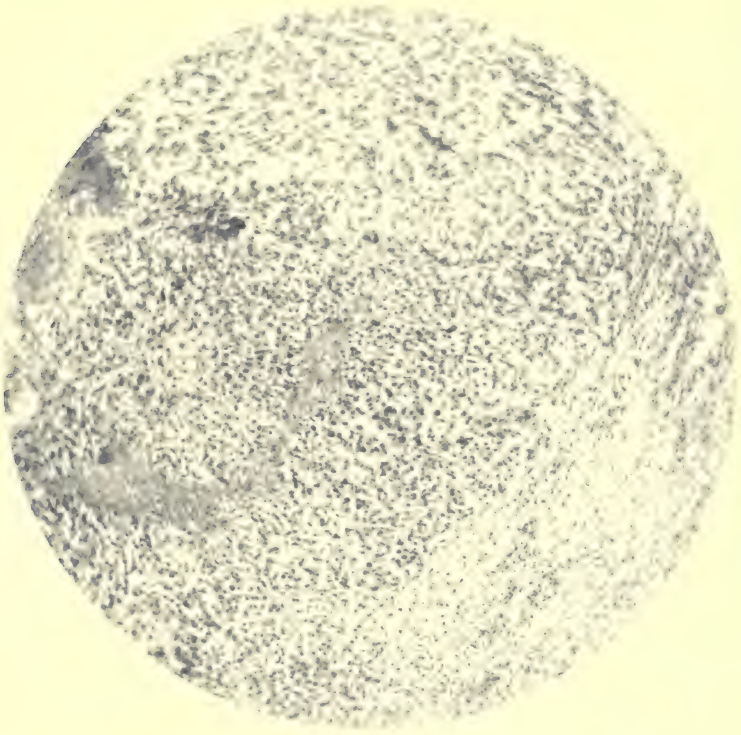

FIG. 1. PRIMARY CARCINOMA OF THE KIDNEY

The junction of the compressed renal tissue and the tumor is shown. Apparently this tumor is derived from renal epithelium. Mouse 1934. × 110.

in large, pseudo-alveoli with much central necrosis, so that the persistence of the tumor cells near the bands of stroma gives in places the impression of a papilloma. Although the growth does not infiltrate the kidney very much, it has no capsule of its own, infiltrates the renal capsule in places, and is undoubtedly

malignant. Evidently it is a tumor derived from the renal epithelium and may be properly designated as a carcinoma, although the term mesothelioma might also be appropriately used. Presumably it is in an early stage of malignancy in view of the relatively small amount of extension of the growth.

2. Solid adenoma arising in bilateral cystic kidneys. Male mouse (9907), age two years, thirteen days. Both kidneys were converted into masses of small cysts of various sizes, resembling the congenital cystic kidneys, although no cysts were found in the liver. Some of the cysts contain colloid masses, and between them are occasional foci of small round cells. There remains more kidney tissue in a functional condition than is usually seen in fatal human cases of congenital cystic kidney, but this tissue is far from normal, the tubules containing many hyaline casts, the interstitial tissue being infiltrated with round cells, and many of the glomeruli being more or less hyalinized. In the lower pole of each kidney was a fleshy nodule about 3 to 4 mm. in diameter. Microscopically these nodules are composed of a solid tissue, made up of groups of large epithelial cells somewhat resembling renal epithelium. About these is a delicate stroma containing some collections of small round cells. The nodule is distinctly encapsulated and seems to be in the renal substance rather than within one of the cysts. In many respects the structure resembles that of the benign ovarian adenomas of mice (47). No abnormalities of importance were noted in the other organs.

3. Adenoma of the kidney (fig. 2). Male mouse (10220), which died with advanced sarcosporidiosis; had marked chronic nephritis, both kidneys being large and nodular, with atrophic areas alternating with areas of swollen and dilated tubules, some of the latter being distinctly cystic. Beneath the capsule of one kidney was a nodule 4 mm. in diameter, with a well defined capsule. It consists of a mass of large epithelial cells with solid cytoplasm, arranged in large cords with a very delicate stroma between them. The arrangement of the cells resembles that of the adrenal adenomas, and a diagnosis of benign hypernephroma might readily be made. The cells are less vacuolated than those

of the typical adrenal cortex, having a denser cytoplasm, and are not dissimilar to the epithelial cells found lining the distended renal tubules; it seems probable, therefore, that this adenoma is derived from renal epithelium. There are some clefts containing

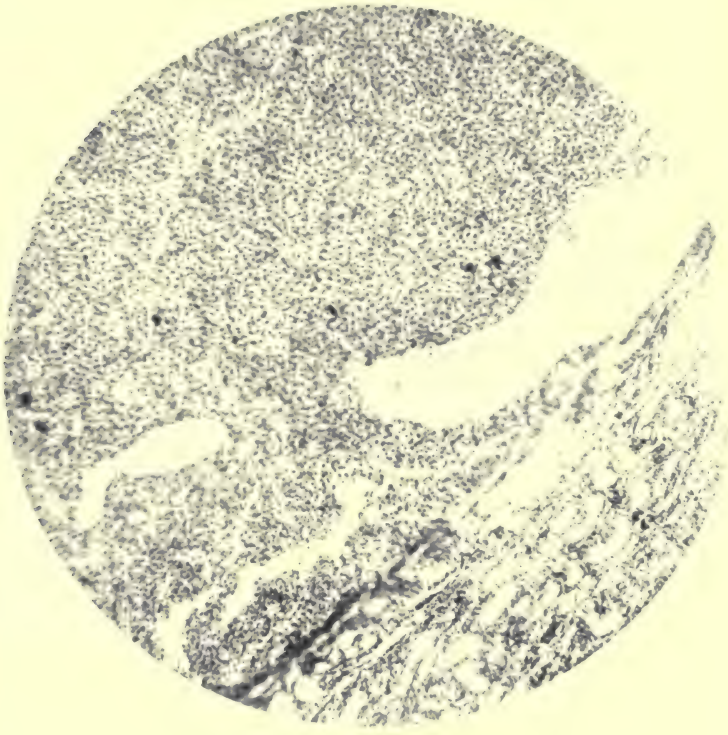

FIG. 2. ADENOMA OF KIDNEY

This somewhat resembles a tumor derived from adrenal cells, but is believed to have been derived from renal epithelium. Mouse 10220. × 65.

colloid material, and numerous calcific granules in the form of calcospherites.

4. Adenoma of the kidney. Male mouse (24073), with a large liver cyst containing a tapeworm, exhibiting in one kidney a solid subcapsular nodule 5 mm. in diameter, with a delicate but definite capsule. This is composed of a solid mass of large

epithelial cells with abundant foamy cytoplasm and small dark nuclei. The cells are in large cords or alveoli with a very delicate stroma between them. There are numerous small foci of calcification of the tumor tissue. The foamy character of the tumor cells, together with their arrangement, suggests an adrenal

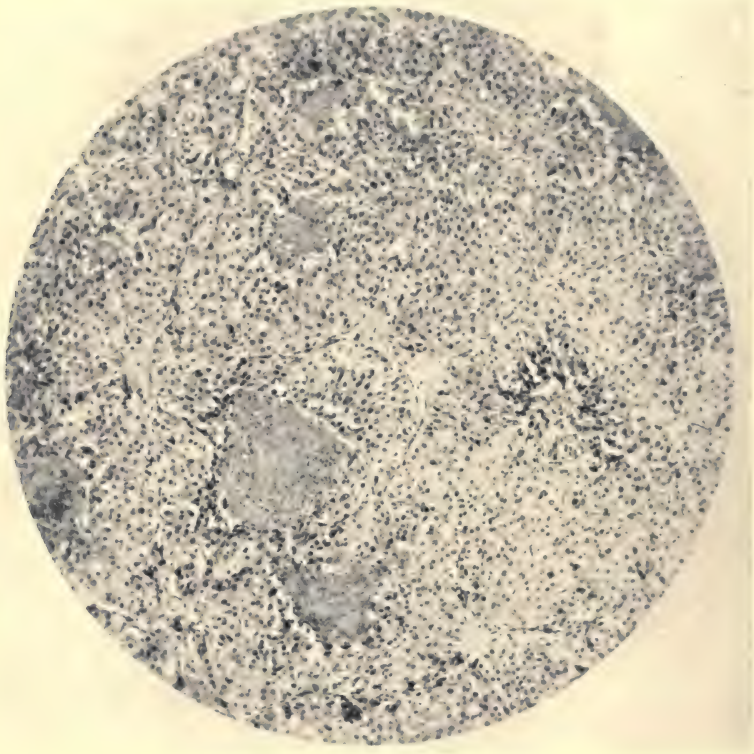

FIG. 3. RENAL HYPERNEPHROMA

This growth entirely replaced one kidney, but produced no metastases; it corresponds in structure to human hypernephromas. Mouse 3639. × 110.

origin for this growth, but the post-mortem changes have so altered the nuclear and cytoplasmic details that this cannot be determined positively. In places there is a tendency to tubular arrangement of the tumor cells, suggesting a renal origin. Outside the tumor the kidney shows an advanced chronic nephritis with many hyaline and fibroid glomeruli.

5. Hypernephroma in the kidney (fig. 3). Male mouse (3639) with no other lesions of significance. The left kidney was replaced by an encapsulated, hemorrhagic mass, measuring 22 x 22 x 18 mm. The right kidney was slightly enlarged and soft, and showed a moderate degree of chronic nephritis. No remains of renal tissue are found in the tumor mass, which presents the typical appearance of a hypernephroma. More than half the mass contains no cells, being composed of the residue of old hemorrhages and necrosis. The living portions consist of large foamy cells arranged in cords and alveoli with a delicate stroma. It is completely surrounded by a capsule which shows no invasion by tumor cells. In all respects this growth corresponds perfectly to the human renal hypernephromas. No metastases can be found.

In the hilum of the right kidney, attached to a large artery, is a nodule about 2 mm. in diameter which, in the center, resembles a small leiomyoma, but about it is a mass of granulation tissue. It bears no resemblance to the hypernephroma and its nature is unknown.

In view of the fact that chronic nephritis is one of the commonest diseases in mice, and appears in forms often quite similar to nephritis in man, it is strange that we have found so few instances of epithelial neoplasms in mice, especially the benign adenomas which are so often found in human kidneys showing chronic nephritis.

SARCOMA OF THE KIDNEY

The diagnosis of sarcoma, always difficult and often unsafe, is particularly dubious in the case of the kidney which presents so many non-sarcomatous growths that resemble sarcoma, and especially in the face of the statement made by Ewing (48): "Birch-Hirschfeld's group of adeno-angiosarcoma, derived from Wolffian remnants, and the lipomyosarcomas remain, however, the only well-defined varieties of renal sarcoma which have been fully divorced from a probable epithelial origin." Nevertheless, since the kidney contains connective tissue it is perfectly possible for sarcomas to arise therein, and in this mouse material we have

several specimens for which only the diagnosis of sarcoma can be made, after excluding all other possibilities. In doing this we have carefully eliminated numerous growths of doubtful character, some of which also may really be sarcomas.[1] These cases are briefly described as follows:

6. Bilateral sarcoma of kidneys. Male mouse (7667), with both kidneys symmetrically enlarged to equal size, about 17 x 10 x 9 mm. They contained little recognizable kidney tissue, and were for the most part infiltrated by a fleshy, pinkish white tissue which involved equally the cortical and medullary portions. No lesions were found elsewhere. Microscopically both kidneys show infiltration replacing about 80 to 90 per cent of the renal elements. The neoplasm is composed everywhere of slightly oval cells, a little larger than lymphocytes, with deeply staining nuclei and very little cytoplasm. No evidence of neoplastic epithelial or mixed tumor elements can be found. This tissue infiltrates between the tubules much as do the cells in leukemic infiltrations, and the capsule is also invaded. The diffuseness of this infiltrative growth and its equal involvement of both kidneys makes it resemble a leukemia or pseudoleukemia, but this diagnosis is untenable in view of the lack of involvement of other organs or lymph-nodes. The diagnosis of sarcoma is made largely by exclusion.

[1] We wish to quote here a statement of the criteria used in our consideration of sarcoma throughout this work as expressed in our paper on Primary Spontaneous Sarcoma in Mice (Jour. Cancer Res., 1917, ii, 1). "We recognize fully the difficulties that attend the differentiation of sarcoma, and for the purpose of this study have excluded every form of new growth concerning the nature of which there seemed any possible room for question. Therefore, we have not included numerous cases in which we think that the growths are probably sarcomatous, and many more in which we cannot be sure that the neoplasm is not sarcoma. On the other hand, the statistical value of our figures is lessened by the fact that we have undoubtedly omitted some growths that are true sarcomas. Our figures represent minimal values only. From the standpoint of investigations in heredity, with which our work is particularly concerned, it is just as undesirable to call a sarcoma something else as to include a granuloma among the sarcomas, and hence the rigid classification adopted in this study of sarcomas is no more satisfactory for our heredity statistics than would be a lax classification that included some growths of doubtful nature. Therefore, in charting the heredity statistics it is necessary to recognize the absence of positive criteria for the differentiation of sarcoma, and to admit the borderline cases with a mark of interrogation to indicate this fallibility."

7. Sarcoma of kidney. Old male mouse (24979), with the right kidney entirely replaced by a tumor, 16 x 12 x 10 mm., which infiltrated the adjacent tissues, including the ureter and the pelvis of the opposite kidney. The regional lymph-nodes were also invaded, and there was a mass in the root of the mesentery about 20 mm. in diameter, which seemed also to infiltrate the pancreas.

Microscopically, the right kidney is found to be almost completely replaced by a growth of large round cells, uniform in size, with very little cytoplasm, but with nuclei much larger than those of lymphocytes. There is no tendency to structural arrangement, the tumor cells infiltrating freely the tissues about the kidney, including the adjacent muscles and the pelvis of the opposite kidney, the ureter of which is surrounded by a mass of tumor. The regional lymph-nodes are replaced by tissue of the same character, but the mesenteric mass is completely necrotic, with few cells resembling those of the tumor. By virtue of its highly infiltrative character and the large size of the cells, this seems to be an undoubted sarcoma. The other tissues showed no evidence of either leukemia or pseudoleukemia.

8. Sarcoma of the kidney. Female mouse (26867) with much sarcosporidiosis, showed a marked enlargement of the left kidney, which was between two and three times the normal size, and infiltrated diffusely with a pink, fleshy tissue. Elsewhere in the body there were no important changes. Microscopically it is found that the enlarged kidney is infiltrated extensively, nearly all the renal elements being replaced by a tissue composed of round and elongated cells, considerably larger than lymphocytes and with more cytoplasm. They tend to be arranged in wide bands, growing out from the blood-vessels, but this arrangement is not constant or well developed. The neoplastic tissue grows out from the pelvis and tends to invade the adjacent tissues and the hilum of the opposite kidney. No similar tissue is to be found elsewhere in the mouse. The probable diagnosis is primary sarcoma of the kidney.

9. Sarcoma of the kidney. Female mouse (27148), with the right kidney much enlarged (18 x 15 x 32 mm.) and consisting of

a firm tissue overlaid with softer portions. Microscopically it is found that the kidney is diffusely infiltrated with a growth consisting of polyhedral cells, smaller than epithelial cells usually are, but with a little more cytoplasm than lymphoid cells. These cells exhibit no structural organization and have replaced all but a few of the original renal elements. About one-third of the tissue is necrotic. A small amount of the neoplastic tissue infiltrates the hilum of the left kidney, which also shows amyloidosis. No similar tissue is found elsewhere in the body, and there are no evidences of diffuse lymphoid hyperplasia or similar conditions. The lung contains a small benign adenoma.

The tumor shows much variation in the size of the nuclei, hyperchromatism being common, and occasional very large cells are seen. Unfortunately there is too much post-mortem change for exact details to be seen. The general character of the growth and the absence of similar changes in other tissues make a diagnosis of sarcoma seem inevitable.

10. Bilateral sarcoma of kidneys, with metastasis in spleen. Female mouse (396), with the uterus enormously distended with fluid because of vaginal obstruction by a seminal mass, and with great enlargement of both kidneys by a uniform white tissue. The right kidney measured 18 x 13 mm., the left 15 x 13 mm. On the anterior surface of the left kidney is a depressed scar. In the spleen there is a tumor nodule, 4 x 5 mm. No other tumor growths were found. The adrenals were normal.

Microscopically, both kidneys are found to be infiltrated diffusely, with replacement of 80 to 90 per cent of the renal tissue by a growth uniformly composed of small oval cells in solid masses, without any particular organization. No similar tissue can be found in any other organ except the spleen, which has a tumor nodule of the same structure as the renal tumor. The adrenals are in contact with, but not invaded by tumor. The tumor forms large masses in the hilum of one of the kidneys, or within the kidney itself. The left kidney is more involved than the right, and probably was the starting point of the sarcoma.

11. Lymphosarcoma of kidney. Female mouse (13124), with two separate mammary carcinomas, and in the left kidney a

nearly spherical mass about 14 mm. in diameter, which was partly necrotic. Microscopically this growth is composed of a dense mass of small round cells without visible cytoplasm, packed closely together without attempt at formation of any structure. It infiltrates the adjacent renal tissue freely, and there is a small amount of infiltration of the hilum of the opposite kidney. The structure is of distinctly neoplastic character, and there is no similar infiltration of other organs, or lymph-node enlargement, such as characterizes pseudoleukemia. The diagnosis of sarcoma, in structure corresponding to lymphosarcoma, is the only one that can be made on the features presented.

12. Lymphosarcoma of kidney. This seems to be similar to case 11, but unfortunately post-mortem decomposition has advanced so far that accurate microscopic study is not possible. Such tissue as remains stainable resembles a lymphosarcoma. The mouse was a female (12533) with no other tumors, and the right kidney was replaced by a soft pink mass measuring 14 x 12 x 12 mm. A small amount of the same tissue invaded the hilum of the left kidney.

MESOTHELIOMA OF THE KIDNEY

In our previous papers on tumors in the ovary (49) and testicle (50) of mice we have called attention to the not infrequent occurrence of tumors composed of polyhedral cells, presenting some characteristics resembling carcinoma and some resembling sarcoma, (figs. 4, 6, 7 and 8) and hence suitably designated mesothelioma, in accordance with Adami. These tumors are characteristic of the urogenital anlage, and hence it is not surprising that growths of the same structure are found in the adrenal and kidney. We have observed the following cases of renal tumors that seem to belong to this group.

13. This is a remarkable case in that a female mouse (21663), when *but one month old* exhibited two independent carcinomas of the mammary gland, and osteosarcomatous growths in the spinal column near the pelvis and in the left fifth rib. It lived but eighteen days more and at autopsy showed its left kidney also almost completely replaced by a tumor measuring 18 x 12 x

10 mm.; the right kidney, similarly involved, measured 10 x 6 x 6 mm. (fig. 4). These renal growths are entirely different in structure from the other four tumors. They are alike and vary in appearance in different parts, some portions resembling

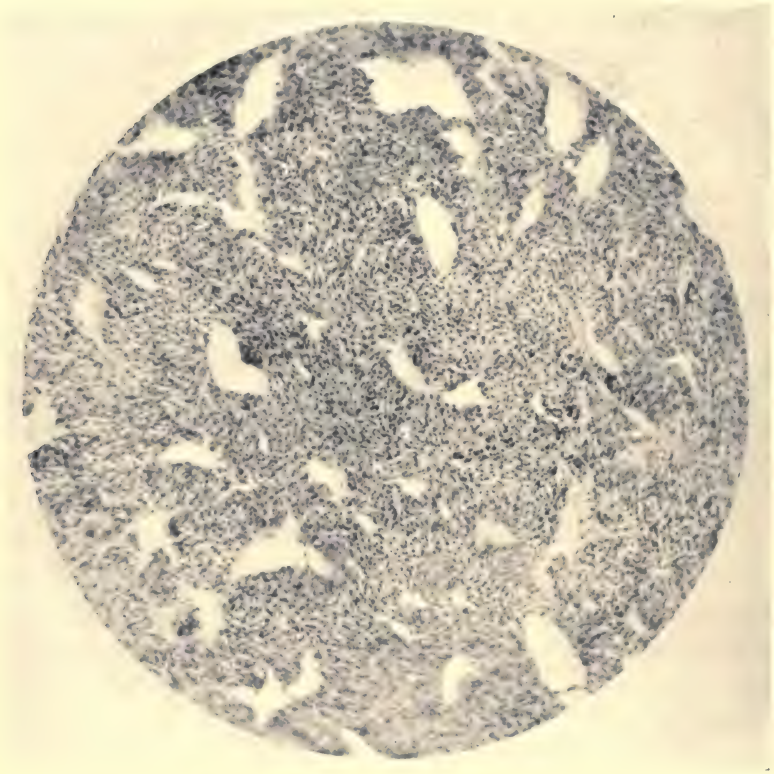

FIG. 4. MESOTHELIOMA OF KIDNEY

This portion of the growth presents a sarcomatous character; other portions exhibit more resemblance to epithelial growth. The tumor was found in a mouse but one month old, with four other tumors, which if not congenital must have developed very soon after birth. Mouse 21663. × 110.

spindle-cell sarcoma while others are composed of larger, more polyhedral cells, arranged in a somewhat alveolar fashion, often separated by highly vascular septa. This structure corresponds to the type of growth often seen in tumors of the ovaries, testicles,

and adrenals in mice, and agrees with the tumors called mesothelioma by Adami and Woolley. Since, except for this case, there have been very few other cases of malignant tumors arising in mice less than six months of age, the occurrence in so young an animal of at least four independent primary growths representing three distinct types of malignant neoplasm, is a most remarable condition, without, as far as we know, a parallel in either mouse cancers or in those of any other animal.

14. Male mouse (10011), with no other lesions of importance, had its right kidney largely replaced by a whitish tumor, forming a mass measuring 14 x 12 x 10 mm. Microscopically the tumor has largely replaced the kidney and has infiltrated the capsule in places. It is composed of small cells with a dark, round nucleus and a small amount of cytoplasm. These cells tend to form bands or pseudo-alveoli in some places, but for the most part the growth is composed of cells in a structureless mass. The left kidney was not involved by the tumor.

15. This seems to be similar to case 14. Male mouse (9779) had an enlarged left kidney without other lesions of note. Microscopically the kidney is largely replaced by a mass composed of small cells slightly larger than lymphocytes and with more cytoplasm, showing no structural arrangement. The main neoplastic mass lies at one side of the kidney, which it infiltrates slightly. The kidney itself shows some foci of round-cell infiltration. Some of these areas slightly resemble the tumor, but probably are not a part of it. There are some small areas of calcification, some scars, and numerous hyaline casts. Postmortem changes are too advanced for more accurate study. The opposite kidney contains no tumor, but there is the same amount of amyloid and calcification.

SARCOMA OF RENAL PELVIS

We have excluded numerous cases in which a retroperitoneal tumor of sarcomatous character has invaded the kidney hilum, but there is one case in which both the gross and microscopical findings distinctly indicate that the tumor had its origin in the tissues of the pelvis itself.

Female mouse (348) had a pale swelling extending downward and inward from the pelvis, composed of tissue of about the same consistency as the kidney and of uniform yellow color. On cross section the pelvis of the kidney was in the center of the mass formed by the tumor and the kidney, which measures 12 x 9 mm. The left kidney was of normal size with a pale area of swelling on the anterior surface. No other changes of importance were found except severe edema of the lungs.

Microscopically the growth is found to lie symmetrically about the pelvis of the kidney and the upper end of the ureter, invading the wall of these structures extensively. From here it passes along the vessels deeply into the kidney, but does not extend far into the cortex. The kidney is about one half as large as the tumor, the tubules being much dilated and the glomeruli more or less hyalinized. The tumor also extends some distance along the renal capsule as a thin layer. As no growths are found elsewhere it is evident that this growth arose in the tissues about the hilum of the right kidney. There is only a small amount of invasion of the left kidney hilum. The growth is composed of large round cells with a delicate reticulum. There are several delicate vessels packed with lymphoid cells, but no other evidences of lymphatic origin in this tumor. The cells of the tumor are polymorphous with considerable cytoplasm, and deeply staining nuclei much larger than those of the ordinary lymph-cell, and usually larger than the nuclei of the renal epithelium. No mitoses are seen. No changes of importance are found in the other organs.

This tumor is much less complex in structure than the cases of sarcoma of the renal pelvis in children described by de Vecchi and Salomon (51).

ADRENAL TUMORS

As mentioned above, no cases of adrenal tumors have hitherto been described in mice. We are able to report a few unquestionable instances of such tumors. Despite the frequency of benign cortical adenomas of the adrenal in man, we have found but one such tumor in mice. It may be recalled that adrenal tumors have not been found frequently in other species of animals.

Cortical adenoma of misplaced inter-renal adrenal rest (fig. 5). Between the kidneys of a female mouse (1921), with marked amyloidosis and chronic nephritis, was found a spherical mass about 5 mm. in diameter, attached neither to the kidneys nor to the intestines. Both adrenals were present at their normal site

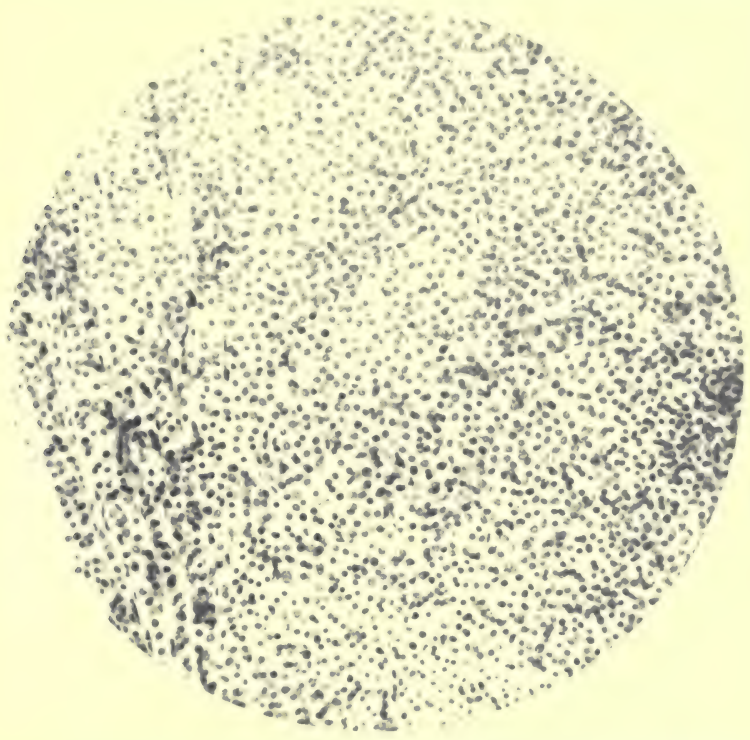

FIG. 5. CORTICAL ADRENAL ADENOMA

The tumor developed in an inter-renal rest of adrenal cortex tissue. A small zone of compressed adrenal tissue is seen at one side. Mouse 1921. ×225.

and were of normal structure and size, except for some peripheral round-cell infiltration.

The tumor nodule is composed of a solid mass of cells resembling those of the adrenal cortex except in lack of orderly arrangement, closely packed together, and flattening out a thin shell of

adrenal cortex, evidently all that remains of an adrenal rest, since no medullary elements are to be found. In all respects this tumor corresponds to the simple adenoma of the adrenal cortex seen in man.

MESOTHELIOMA OF THE ADRENAL

This seems to be the commonest tumor of the adrenal, as also of the testicle, of mice, and it is quite impossible to distinguish on the basis of microscopic appearance between the mesotheliomas arising in the different organs derived from the urogenital anlage. The cases in which the diagnosis seems certain are the following:

1. Male mouse (10390) had in place of the right adrenal a spherical mass 5 mm. in diameter, which is completely encapsulated and does not involve the kidney. No other nodules or findings of importance elsewhere. Microscopically the nodule contains no remains of adrenal tissue, but consists of a solid tumor made up of masses of large cells with considerable cytoplasm and large oval or spherical nuclei, arranged in atypical alveoli or broad bands with a very small amount of stroma containing thin-walled blood-vessels. In numerous places the capsule is infiltrated with tumor cells and there is some invasion of the areolar tissues about the adrenal, but no invasion of the adjacent kidney. This seems to be a typical mesothelioma of the adrenal in an early stage of malignancy.

2. Mesothelioma of adrenal with peritonea' metastasis. Female mouse (12744), with abdomen greatly distended by a bloody exudate, presented at the site of the left adrenal a whitish mass about the size of the kidney. All through the abdominal cavity are masses of partially necrotic whitish tissue, especially attached to the liver and uterus, which do not seem to be involved by this growth. There are enlarged retroperitoneal lymph-nodes, but elsewhere no lymphatic involvement. There are no tumors in the lungs.

Microscopically the tumor shows the usual features of the typical mesotheliomas, as described in the other cases, with slight tendency to alveolar arrangement. It does not seem to have

infiltrated or produced metastases in the viscera. The right adrenal is surrounded, but not invaded, by the tumor and seems normal. The retroperitoneal nodes are replaced entirely by tumor tissue.

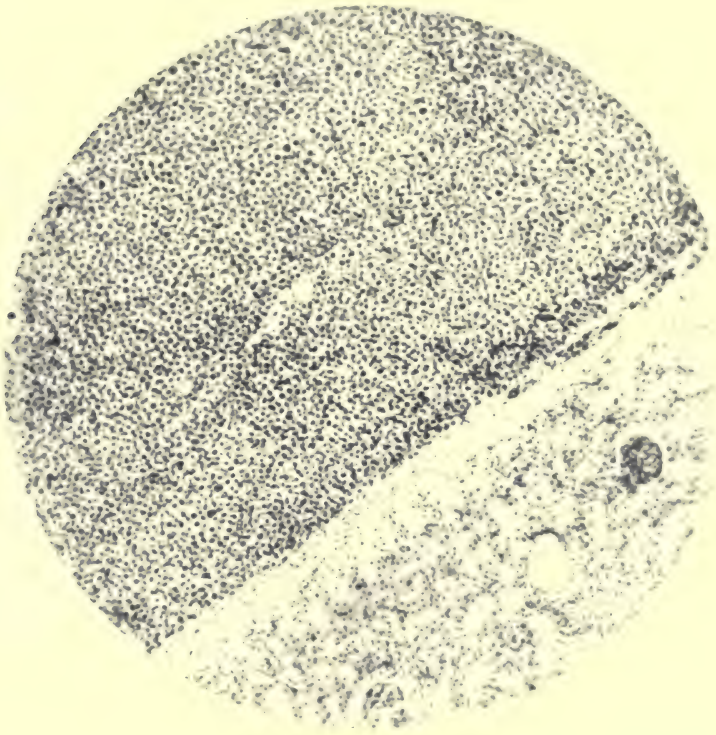

FIG. 6. MESOTHELIOMA OF ADRENAL

This growth surrounded the lymph nodes and was widely disseminated through the perirenal tissues. Mouse 7699. × 110.

3. Bilateral malignant mesothelioma of adrenals (fig. 6). Female mouse (7699) with much subcutaneous edema, showed numerous enlarged subcutaneous lymph-nodes, some red and some pale, the largest measuring as much as 8 x 10 mm. There was a milky fluid in the peritoneal cavity, presumably because of pressure of enlarged lymph-nodes on the thoracic duct, for

the retroperitoneal nodes were greatly enlarged, up to 6 x 12 mm. The mesenteric nodes were also enormously enlarged, one mass at the root of the mesentery measuring 40 x 20 x 20 mm. Both adrenals were greatly enlarged, each being about the size of the kidney, which was adherent to the adrenal but not enlarged or infiltrated by neoplastic tissue. The uterus seemed to be infiltrated by tumor. The liver was not affected, and the lungs showed only a single small nodule, although there was some tissue increase in the upper mediastinum; both lungs showed a bloody edema, and there was a bilateral hydrothorax.

Although the gross appearance suggested a general lymphosarcomatosis or pseudoleukemia, yet the microscopic structure is of an entirely different character. Everywhere the neoplastic tissue presents the same appearance, being composed of a solid growth of cells with considerable cytoplasm, so that they look much like epithelium. The nuclei, which vary greatly in size, are much more solid than those of epithelial cells usually are. Mitotic figures are abundant. The invaded lymph-nodes are largely replaced by tumor cells, which are also found in the lymph-vessels of the lungs, but not in the liver. Both adrenals seem to be entirely replaced by the tumor, which lies upon the capsule of the adjacent kidney without any invasion of this organ. There are some areas of hemorrhage, but not much necrosis. The cells show no attempt at definite arrangement, but simply form a solid mass with numerous, poorly defined blood spaces.

This tumor resembles in structure other growths found arising in the urogenital anlage, and in view of this and of the complete replacement of both adrenals it is most probable that it did arise in these tissues, although the extensive lymph-node involvement is unusual in adrenal tumors. The structure is not essentially dissimilar to that of the adrenal tumor 10390, except for the amount of extension.

UROGENITAL MESOTHELIOMAS OF UNCERTAIN ORIGIN

Because of the identity in appearance of mesotheliomas from all organs originating in the urogenital anlage, it is not always

possible to decide the place of origin of some tumors which involve two or more of these organs, as shown by the following cases.

12307. Mesothelioma of either adrenal or ovary. It is not possible to determine the origin of this tumor, which we have described in our paper on tumors of the ovary in the following words:

> The abdominal cavity shows several nodules whose exact origin is difficult to determine as the mate has partly devoured the body. The right ovary is, however, easily distinguished. It measures 18 x 12 x 12 mm. What seems to be the left ovary is 10 x 8 x 6 mm. There are 8 other similar nodules in the abdominal cavity, one being in the position of the left adrenal, measuring 10 x 8 x 8 mm. The other nodules are apparently in the mesentery. One lobe of the liver is converted into a tumor nodule 14 x 10 x 18 mm., irregular and lumpy in outline, pink in color.
>
> The tumor shows everywhere the same structure, consisting of irregular alveoli composed of large cells with abundant cytoplasm with well defined borders and deeply staining nuclei. Mitotic figures are numerous. The character is that usual to mesothelial growths. The ovary cannot be positively identified, but one mass exhibited in the capsule structure suggests compressed ovarian tissue with degenerated ova. In all respects this tumor is identical with the malignant ovarian tumors just described.
>
> It seems probable that this tumor arose in the ovary which exhibited the largest growth, but it is not possible to exclude the adrenal as the primary site.

The malignant tumors of the adrenal, ovary, and testicle commonly exhibit identically the same histological picture as that seen in this case.

We have also observed two other cases, described in the paper on ovarian tumors in which we cannot state whether the renal growth was primary or secondary.

12876. The left kidney contained a mass of pink, fleshy tissue, 18 x 14 x 14 mm. The right kidney, which was slightly enlarged, contained no tumor. The right ovary consisted of a pinkish tissue resembling that in the kidney, and measured

12 x 8 x 8 mm. In the mesentery was a similar, slightly paler mass, 16 x 8 x 8 mm. The retroperitoneal and subcutaneous nodes were not enlarged and no nodules were found in the lungs.

Microscopically the tumor is alike in all three places, consisting of a diffuse infiltrating growth of large round cells, which also invade the connective tissues about the kidney and ovary. It does not at all resemble the typical ovarian tumors, being apparently a round-cell sarcoma. We have no way of telling which of the three tumors was primary. The next case presents similar difficulties.

26. This mouse had a tumor mass about 8 x 10 mm. in the upper portion of the liver, with other smaller nodules near it. A similar small nodule was found in the right kidney. The right ovary was enlarged to two-thirds the size of a kidney, and was solid. Microscopically all these growths are composed of round cells, apparently a round-cell sarcoma. It is impossible to say which growth was primary.

Among several tumors arising in the retroperitoneal tissues, mostly of sarcomatous type, are a few of a structure identical with the characteristic mesothelioma type of growth that arises in the urogenital anlage. In the two cases described below this character of growth was so marked that it seems probable that the tumors have arisen in some misplaced embryonic rest, since the organs of this series were distinctly not the starting point of the growths. Numerous tumors of this sort have been described in man.

(22380) Malignant retroperitoneal mesothelioma with extension through the body wall. Male mouse presenting externally a subcutaneous tumor, involving the left hip and extending to the anus, the external measurements being 30 x 25 x 25 mm. When the body was opened it was found that this mass was an extension of a huge mass (40 x 20 x 18 mm.) which had surrounded the left kidney and pushed it to the ventral midline, invaded the spleen and pushed it mostly to the right of the midline by formation of a mass that measured 20 x 15 x 13 mm., and extended downward through the pelvic cavity where it surrounded the rectum and seminal vesicles and broke out through the

body wall. There was slight infiltration of the lower pole of the right kidney. The liver was slightly enlarged and mottled with areas of infiltration. There was some tumor in the lower part of the mediastinum, and the lungs show many small spots of neoplastic tissue. There seemed to be no involvement of the

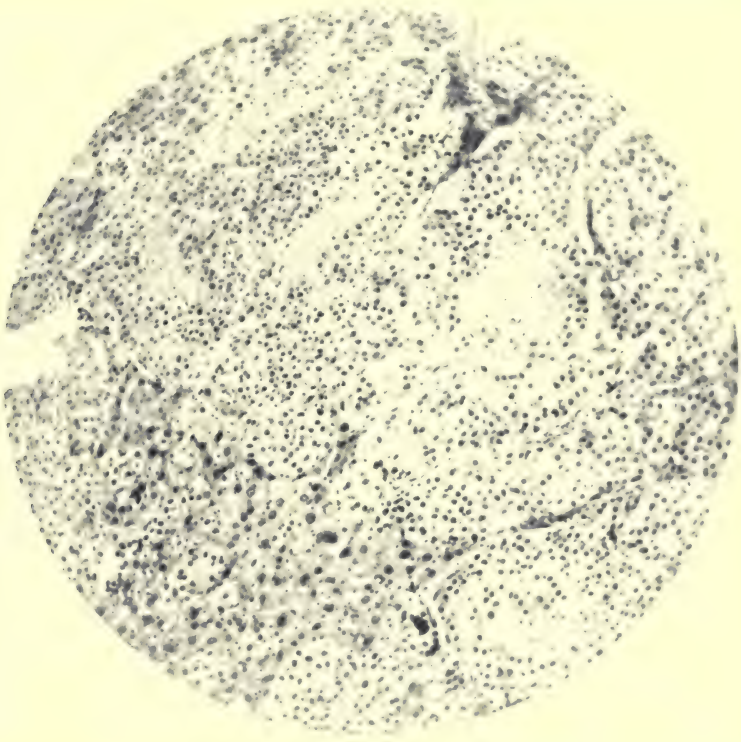

FIG. 7. SECONDARY MESOTHELIOMA IN LIVER

The primary growth was either in the adrenal or in a retroperitoneal embryonic rest; it infiltrated the body wall and retroperitoneal tissues, and produced innumerable metastases in the lungs and liver. This section shows the masses of tumor cells compressing and replacing the larger liver cells. Mouse 22380. ×110.

lymph-nodes, either subcutaneous or abdominal. The testicles were not involved.

Microscopically the tumor everywhere consists of masses of large cells with much deeply staining cytoplasm, somewhat resembling liver cells, with a slight tendency to arrangement in

cords or bands. Where the tumor invades the liver it can be seen that the cells are a little smaller and paler than the liver cells (fig. 7).

The growth infiltrates the liver widely, invades the muscle of the body wall, invades the renal capsule but not the kidney,

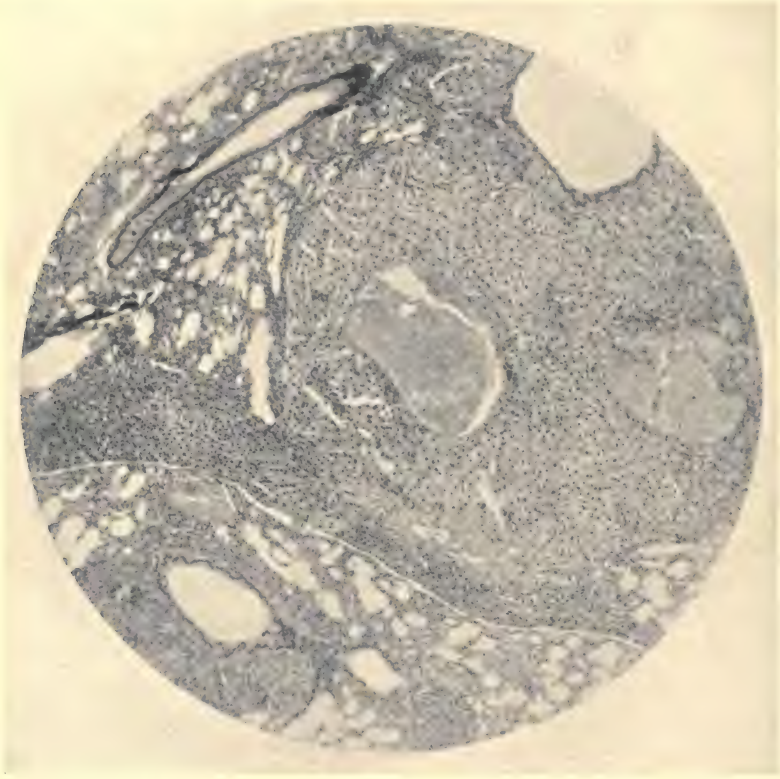

FIG. 8. SECONDARY MESOTHELIOMA OF LUNGS

From the same case as figure 7. The section shows the extent of involvement of the lung. Mouse 22380. × 60.

infiltrates the abdominal sympathetic ganglia, surrounds densely the rectum and spreads into the seminal vesicle, invades the spleen, and in the lung appears as multiple tumor cell emboli within the vessels and as large tumor nodules largely replacing portions of the lung (fig. 8). The left adrenal was found entirely embedded in but not infiltrated by the tumor.

The microscopic appearances are identical with those usual in malignant adrenal tumors, but the adrenal was not involved; and in view of the retroperitoneal origin, the growth may be presumed to have arisen in an embryonal rest of the urogenital anlage.

(9979) Malignant retroperitoneal mesothelioma infiltrating kidneys. Small female mouse with extensive infiltration of the right thigh by a typical spindle-cell sarcoma, and with a mass above the left kidney, about as large as the kidney itself, which was infiltrated by the growth. The entire mass measured 20 x 14 x 10 mm. The right kidney was not so much enlarged. The growth seemed to have arisen at the site of the left adrenal, and to have pushed the kidney forward. The liver was invaded by tumor and enlarged. No metastases were found elsewhere. Microscopically the tumor about the kidney bears no resemblance to the spindle-cell sarcoma of the thigh, being composed of a mass of polyhedral cells arranged in solid masses, with a slight tendency to form bands or cords. The nuclei are not much larger than those of lymphocytes, but the cells have much more cytoplasm. The chief mass lies above and behind the left kidney, which shows considerable invasion through the capsule and about the blood vessels. There is a similar invasion of the right kidney but much less tumor about it. The right adrenal cannot be located; the left is free from tumor although there is a necrotic area between it and the kidney. The liver shows a large nodule of the same sort of tumor.

SECONDARY TUMORS OF THE KIDNEYS AND ADRENALS

The mouse kidneys seem to be extremely insusceptible to metastatic invasion by tumors. In our entire series of primary tumors of mice, now in the neighborhood of 5000 cases, of which the predominating form is carcinoma of the mammary gland, we have never met with a metastatic growth from one of these tumors into the kidneys, with one possible exception, despite the frequency of pulmonary metastates, which often replace most of the lung. The only metastatic carcinomas of the kidney that we have seen have been found

in four cases of primary carcinoma in the lung (3098, 11777, 12373, 14242). A photograph of the first of these four cases appears in our paper on lung tumor (52). These carcinomas of the lung are especially likely to produce extensive metastases, and the suggestion of some authors that these pulmonary growths in mice should not be included among the true tumors is evidently based on ignorance of their character. The fact that they have furnished our only instances of metastatic carcinoma in the kidney is sufficient evidence of their true neoplastic character and the high malignancy of some types.

Although we have seen many instances of widespread metastasis in sarcoma, the kidneys are almost immune from vascular sarcoma embolism. In our series of 87 cases of sarcoma in mice, although 23 showed metastasis but one hematogenous secondary nodule was found in the kidney, this coming from a mediastinal sarcoma (11791). Since then we have seen one other case of vascular metastasis of sarcoma into the kidney, from a sarcoma of the uterus (12058), with a large nodule of the same structure almost replacing the lower pole of the right kidney, and metastasis in the right ovary. Even the widespread growths of small round cells, which resemble the condition called lymphosarcomatosis in man, seem to affect the kidney but little, for among a considerable number of such cases we have but one with a distinct metastatic nodule in the kidney (7572), although several cases show extension from retroperitoneal metastases into the capsule, and then into the kidney. On the other hand, the kidneys exhibit extensive diffuse infiltration in leukemia and massive perivascular growths in pseudoleukemia in mice as in man.

The malignant retroperitoneal growths, most of which resemble lymphosarcoma, and the malignant tumors derived from the adrenals, commonly invade the kidney by direct extension into the hilum, often very extensively. The sarcomas primary in one kidney also tend to spread into the hilum of the opposite kidney. This ready invasion of the hilum of the kidney is a point of similarity of human and mouse neoplasms.

No instance of tumor metastasis into the adrenal has ever been observed, except possibly in the few cases of widespread mesotheliomatous growths invading both the ovaries and adrenals, the origin of which is uncertain.

SUMMARY

In a series of 33,000 autopsies on mice of the Slye stock, dying natural deaths at all ages, but as far as possible living out their natural span of life, there have been observed the following cases of true primary neoplasm arising from renal or adrenal tissues: First, from the kidney, 16 tumors, classified as follows: 1 carcinoma, 3 adenomas, 1 hypernephroma, 7 sarcomas, 3 mesotheliomas, and 1 sarcoma of the renal pelvis. Second, from the adrenal, 4 tumors, as follows: 1 cortical adenoma from a misplaced inter-renal adrenal rest, 3 mesothelial tumors. Third, five cases of tumors of the mesothelial structure characteristic of urogenital anlage neoplasms, but of which the exact origin could not be determined because of their widespread growth at the time of death. As these 25 tumors occurred in 33,000 mice presenting not far from 5000 other tumors, they are evidently uncommon tumors of mice, at least in this particular stock.

It will be noted that in this series there has been no instance of a mixed renal tumor of the Wilms type, which is so common a type of renal tumor in man and apparently also in swine. Although inflammatory conditions are very prevalent in the kidneys of mice, epithelial tumors are rare, and especially to be noted is the absence of even a single case of typical malignant hypernephroma, although one benign growth of this type was found. No epithelial tumors of the renal pelvis were found, although there was one case of sarcoma that seemed to take its origin in the pelvis.

Several instances of malignant retroperitoneal tumors have been observed, mostly of sarcomatous structure, which usually invade the kidney. These have not been included in this series, except two cases in which the structure resembled that of the

mesotheliomas, suggesting that the tumor had its origin in misplaced rests of the urogenital anlage.

Secondary tumors have never been found in the adrenals, and but rarely in the kidneys. Although this series includes at least 3000 cases of mammary carcinoma, often with widespread metastases in the lungs, we have never seen a secondary carcinomatous growth in the kidney. The only secondary carcinomas of the kidney as yet observed are four cases in which the primary carcinoma was in the lung, thus establishing the true neoplastic nature of these lung growths. In but two cases have metastatic sarcomas been seen in the kidney, if we exclude the numerous cases of invasion of the kidney by direct extension from para-renal growths.

As to sex: In the entire group of renal and adrenal tumors, we have equal numbers in males and females, agreeing with the observation made on other tumors in mice that, in tumors not peculiar to the sex glands, there is usually little difference in the incidence in the two sexes.

Differing from the tumors previously studied, coincidence of other tumors with the renal and adrenal tumors is uncommon. One mouse in this series had a spindle-cell sarcoma of the thigh. One mouse had a small, benign papillary adenoma of the lung. Only two mice had a mammary carcinoma, and one of these (21663) was a remarkable case, for this animal, when but one month old, was found with two independent mammary carcinomas, and with osteosarcomas in the spinal column and in a rib. It lived eighteen days more, and at autopsy there was also found a mesotheliomatous type of growth involving both kidneys. Except for this unique case there have been practically no instances of malignant tumors in mice less than four months of age, and few under six months. Most of the renal sarcomas occurred between the ages of seven months and one year, which is somewhat earlier than the usual time of appearance of epithelial growths; this, of course, corresponds to experience with human neoplasms.

The epithelial renal and adrenal tumors furnished no illustration of metastasis, but in three cases of sarcomatous or meso-

theliomatous growths there was noted involvement of the adjacent lymph-nodes; in two there were pulmonary, in two hepatic, and in one splenic metastasis, and in one case there were numerous peritoneal growths. The mesothelial type of growths produced the most extensive metastasis and the most widespread infiltration of the body wall.

A review of the literature on renal tumors throughout the animal kingdom, which constitutes the introductory part of this article, discloses but six other cases of renal tumors in mice, all epithelial, and no other adrenal tumors.

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BIOLOGICAL EVIDENCE FOR THE INHERITABILITY OF CANCER IN MAN[1]

STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

EIGHTEENTH REPORT

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The biologic problem of the nature and inheritability of spontaneous cancer has been under study in this laboratory for the past twelve years and for ten years the results of that study have been in published form. During this time an increasingly broader and deeper mass of facts, always perfectly consistent, has steadily been obtained. These facts have been presented before scientific societies year by year, and have been published with masses of exact data (1–16).

One of the facts demonstrated in these studies is the inheritability of the tendency to spontaneous cancer, with its strong evidence against the probability of cancer being a specific germ disease.

The more superficial aspect of this demonstration, viz., its practical meaning to the human race, is the phase of the work which has mainly impressed the pathological and medical world. The more profound and biologic aspect of the demonstration has, for the most part, failed to make an impression, and there is a rather wide-spread medical opinion today, backed by some published pathological opinion represented, for example, by

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Ewing in his *Neoplastic Disease*, that a demonstration of the inheritability of cancer in mice (which is now quite generally conceded) has no bearing upon the question of the inheritability of cancer in man.

Let us for example examine the section on heredity in the second edition of Ewing's *Neoplastic Diseases* (17). This section scarcely permits of constructive criticism, for in one paragraph the author admits that the cancer tendency is inherited in certain human families, and in the next he vigorously states that "this fact in no way justifies the assumption that hereditary influences prevail for cancer in general." This is as if he should state that although black hair is hereditary in some families of negroes, it is not inheritable in the rest of the negro race where it also uniformly occurs.

Again, referring to laboratory studies in the inheritability of animal cancer, Ewing states that Tyzzer has demonstrated the hereditary theory for one family of mice, but he nowhere admits the general application of the laws of heredity, as, for example, to the rest of Tyzzer's cases or to the 4000 cases in the Slye stock, where cancer also uniformly occurs in exact accordance with the laws of heredity. This is as if he should say that albinism is inheritable in one family of the Tyzzer stock of mice, but that there is no justification for supposing that albinism is inheritable in the rest of the Tyzzer stock; or in the Slye stock where it has uniformly occurred in about 20,000 cases in exact accordance with the laws of heredity.

Again, on page 107 Ewing states that "human statistics of cancer heredity are worthless," but in the next paragraph he states that a pronounced hereditary predisposition to the disease does exist in the human families mentioned by Warren, Broca, etc.; and he finally closes the discussion by stating that "we must go into the field of human statistics" (which he has above pronounced worthless) "in order to determine by observation just how far any hereditary influence proves effective in the causation of tumor."

On account of such published opinion, totally inconsistent as it is, and the prevalence of similar ideas in many sections of the

medical profession, it has seemed advisable at this time to present the more fundamental aspect of the matter, and to point out what is the bearing upon the human cancer problem of these studies in the inheritability of animal cancer.

Under every smallest instance of behavior in the organic world which has ever been studied, there is biologic law. It is contrary to every biologic analogy to say there is no law for any given type of organic behavior, because we have not yet discovered it. There is a multiplicity of these laws and their ramifications, of which, in all probability, we have no knowledge whatever. But there is one of these biologic laws of which we have the fundamental facts; that is, the law of heredity. And having the facts of this most fundamental and most potent of all biologic laws, we continue in actual practice completely to ignore it.

What is heredity? If you consult the dictionary you will get some such definition as this: "Heredity, the law according to which plants and animals inherit and transmit from generation to generation certain characteristics or tendencies." But if we give it its full biologic definition, we must say: Heredity is the force which makes and holds together the genus and the species. It determines that birds shall have wings and a special chest capacity for flight; that they shall have a bill, wide vision on all sides, etc., and for the specific bird it determines a certain plumage and a certain song, as of the robin, or the blue bird, or the thrush.

It determines that the frog shall have a special breathing apparatus to operate on land or in the water; and for the specific frog, as the bullfrog, that he shall have a certain coat color and pattern, a given size, a given call.

It goes even deeper than this, and determines that the human embryo, beginning with a single cell, like any unicellular animal or plant, divides in the same way, and in its complex cell division and differentiation recapitulates in hurried fashion the history of organic evolution.

Why? Let me here state what I conceive to be the biologic law of heredity, the law which underlies all life: *That which goes into the germ plasm, must come out in the offspring.* We must

conceive of this simple law as being as iron-clad and as immutable as a law of physics: action and reaction are equal and in opposite directions; or a law of chemistry: hydrochloric acid on zinc will produce zinc chloride. If you pour acid on metal you will get a given reaction whether you want it or not. What is put into the germ plasm will come out in the offspring, whether we want it or not—a law of nature, immutable, deaf to entreaty.

Now the unique feature of natural law is that we cannot break it. We can study it, learn to understand it and work with it, or we can continue to ignore it and combat it and be broken by it, but we cannot break it or change it; only so does the organic world hold together.

It is a general law, this law of heredity; not one law for a mouse and another for a man, another for a guinea-pig and another for a geranium, but one common law of heredity. What goes into the germ plasm comes out in the offspring, whether it is the seed of a geranium, the ovum of a guinea-pig, or of man.

And in the progress of evolution we see the constant and unbroken control of heredity, so that man, the latest product (to date) of evolution, starts with a single cell, recapitulates in his embryonal growth the history of organic evolution, and in his turn sets off the single cell (the germ plasm) made of the stuff he received from his ancestry and puts into it the identical material. This single cell (the germ plasm) in its turn again divides and in its embryonal development briefly recapitulates organic history, and in time becomes the finished example of the species; made of the material received from his ancestry—in his general build, in his length of leg, in his shape of nose, in his hair color, in the kind of kidney he has, and the kind of liver he has, and the kind of lungs and heart he has; in the kind of epithelium he has, and the kind of connective tissue, and the kind of endothelium. He starts with a vague nose shape, but it will grow into the nose shape of his ancestors; he starts with tiny legs, but they will grow to the inherited length. He inherits a liver which will in time react like the livers of his ancestors to the same causes. He inherits a type of epithelium and of connective tissue which will in time react like the epithelium and the connective tissue of his ancestors to the same causes.

So much for the general, the immutable law of heredity: What goes into the germ plasm comes out in the offspring.

How does heredity work? As long ago as 1865 Mendel worked out with green peas the best study of the method of heredity that we have ever had. Later, and following him, Cuenot and others worked it out with mice, and it worked out with mice exactly as it worked with peas. Now it is vastly farther in the scheme of evolution from peas to mice than it is from mice to man. Mice are mammals like man, their structure is like man's —a head, a trunk, four limbs. Their organs are like man's arranged in the same relation to each other, made out of the same types of materials, functioning in the same way for the maintenance of the organism. If we cut a mouse's arm it bleeds like man's, and then regeneration sets in as it does in man, the edges draw together, the epithelium proliferates, scar tissue is formed, which eventually either in part or wholly is absorbed—a process identical with that of man's tissues, functioning like those of a man, just as the geranium stock does if you cut it. Why? The law of heredity, transmitting a type of protoplasmic behavior down the full line of evolution; similar tissues functioning in the same way because they were derived from a common ancestry. If we do not accept this, we must discard the theory of evolution, for this is the heart of the theory of evolution.

Now the method of heredity as worked out by Mendel, and repeated with mice by Cuenot is this: when a pure-bred grey house mouse is crossed with a pure-bred albino, the first hybrid generation will all be grey. That is, the tendency to pigmentation is dominant over the tendency to the lack of pigmentation. Now if we mate two of these first generation hybrid greys (heterozygotes) we shall get in the second hybrid generation some pure-breeding greys (dominants), some heterozygous greys, and some albinos (recessives), in the proportion of one to two to one. These dominant greys, if bred together or hybridized with other dominant greys similarly derived, will breed true. The recessive albinos, whether inbred or hybridized with other pure-breeding albinos, will breed true. The heterozygous greys, whether inbred or hybridized with other heterozygous greys simi-

larly derived, will again yield the three types, dominant greys, heterozygous greys, and recessive albinos, in the proportion of one to two to one (see chart 2, p. 118).

Again, if we cross a pure-bred albino with a heterozygous grey we shall get, in the first hybrid generation, recessive albinos and heterozygous greys in the proportion of one to one. These albinos will breed true whether inbred or hybridized with other pure-bred albinos. The heterozygous greys, in this case also, whether inbred or hybridized with other similarly derived heterozygous greys, will give the same three types: dominant greys, heterozygous greys, and recessive albinos.

Again, if we cross a dominant grey with a heterozygous grey, the first hybrid generation will give dominant greys and heterozygous greys in the proportion of one to one. The dominant greys will breed true, and the heterozygous greys will again give the same three types.

Why do these characters behave in this way? For that they do is certain. What is the secret of the method of heredity? If we mate two pure-bred albinos the offspring will all be albino; i.e., there will be a complete lack of the pigment-making mechanism. Pigment is an absent character in these individuals. It did not go into their germ plasm and they cannot transmit it to their offspring. If only albino mice are allowed to breed, the pigment-making mechanism will be lost for mice, and cannot be recovered, and there will thereafter be only albino mice. Albinism is a recessive. Pure-bred albinos cannot transmit the dominant.

Now if we mate the same pure-bred albino into whose germ plasm no pigment-making mechanism went, with a grey house mouse into whose germ plasm the tendency to pigment-making mechanism did go, we shall get in the first hybrid generation heterozygous greys, i.e., pigment-making is dominant over the lack of pigment-making, therefore the mice are grey. But into their germ plasm went from one side the absence of the pigment-making mechanism (as a unit character) and from the other side the presence of the pigment-making mechanism (another unit character); so since both these unit characters went into their

germ plasm, both of these characters will come out somewhere in the offspring—the law of heredity.

All efficient study of heredity is the study of the behavior of unit characters. Only we must be sure that we have analyzed our characteristics into unit characters, which segregate out and are transmitted as such, like albinism and pigmentation, spotting and self-color, etc. And conversely, when in the study of heredity we have found a character which does segregate out and is transmitted as such, we have found a unit character, which will behave in accordance with the immutable law of heredity for the unit character. A unit character is then to heredity what an electron is to chemistry; incapable of analysis, it segregates out and is transmitted as such.

Now when we are dealing with a complex organism like a man or a mouse, there is a multiplicity of these unit characters which have gone into his germ plasm from his ancestors, and which will get into all possible combinations. For example, a tendency to a heavy and a tall skeleton; a tendency to a particular length in the limbs; a tendency to blackness of hair with a tendency to curliness of hair; a tendency to a straight nose with a tendency to a large nose; a tendency to a certain kind of liver, which will tend to a certain type of epithelium, with a tendency to a certain type of behavior, etc.—all unit characters.

Every organism, then, is a synthesis of unit characters which cannot be correctly manipulated, or interpreted in experimental work with accurate results, until it has been analyzed into its component unit characters. Until the truth of this fact has come home to the experimental biologist, pathologist, bacteriologist, physiologist, and student of therapy, our results are certain to be invalidated by artifacts. This applies in all experimentally produced cancer; whether by grafts, or by artificial chronic irritations like painting with coal-tar products and feeding with nematode and tapeworm larvae, just as it applies everywhere else in experimental research. First by analyzing our stocks into unit characters, we must learn what portion of the result is produced by nature without reference to the experiment, and what is the experimental residuum.

With these things in mind, I undertook the study of the heredity behavior of cancer, to find out the nature of cancer, partly in order to learn how to get rid of a hideous disease, and partly for the light it must throw on general biologic problems of all tissue behavior. I proceeded to study the inheritability of cancer in exactly this classic way, that is by making a biologic analysis of stock, without which no stock is of any value for conclusions concerning heredity, or practically anything else.

How do we make a biologic analysis of stock? Breed it out, so as to find out what unit characters went into its germ plasm. It then becomes a stock made up of analyzed individuals, whose hereditary potentialities are known and whose effect in any cross can be predicted.

On the other hand, if you buy animals in the market and proceed to use them for even a simple experiment, they are worthless until they are analyzed, because some of them may be pure-bred and some of them inevitably will be heterozygous, and they will not behave alike in any given experiment, since they have not the same unit characters. You have no biologic control in the experiment until each animal to be used is analyzed.

I proceeded, then, to study the inheritability of cancer in this way, making a biologic analysis of the stock by hybridization and inbreeding, and I found that *equally by the method of hybridization and by inbreeding*, if you mate two mice with carcinoma of the lung (primary or secondary) you can extract from them a strain of 100 per cent lung tumor mice; or by mating two mammary gland carcinoma mice, a strain of 100 per cent mammary gland carcinoma can be extracted from them. That is, both in hybridization and in inbreeding, cancer behaves like a unit character; in other words, it segregates out and is transmitted as such.

I tried, also, further tests of hybridization between cancer mice and absolutely non-cancer mice. By a cancer mouse we mean a mouse whose ancestry had cancer, into whose germ plasm the tendency to cancer entered, and who himself has cancer. By a non-cancer mouse we mean a mouse which came from wholly non-cancerous parentage, into whose germ plasm there

went the tendency to the absence of cancer, and who cannot transmit the cancer tendency to his offspring. They are analyzed individuals, both of them, whose heredity behavior can be predicted.

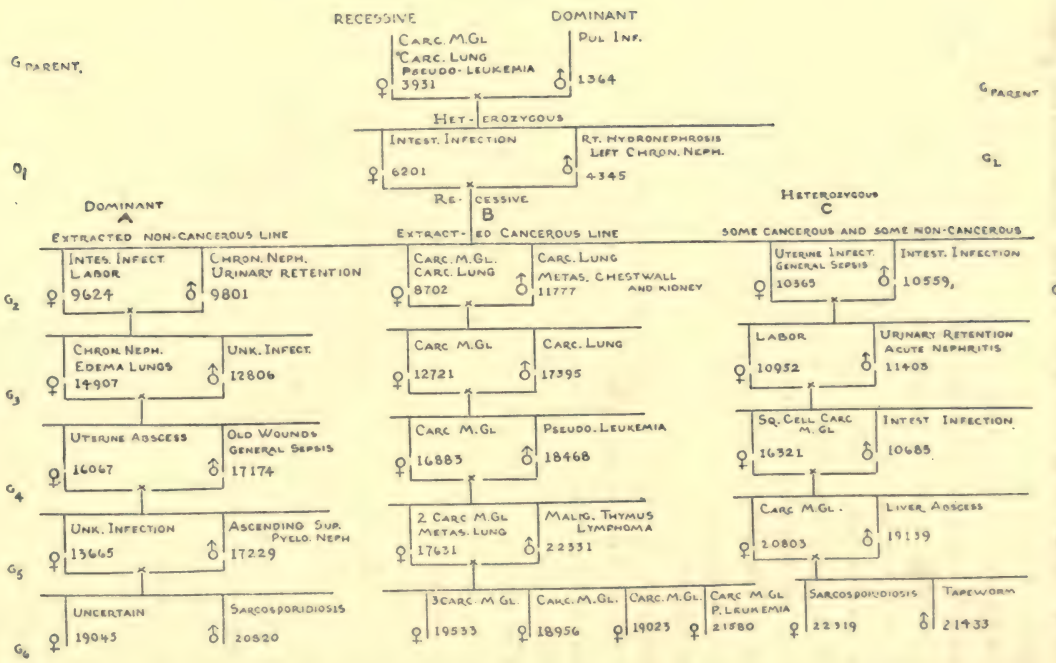
If I mate such a non-cancer mouse with a cancer mouse, into the common progeny of these two there go, first, a tendency to cancer, and second, a tendency to the absence of cancer; and the first hybrid generation can, and infallibly does, transmit some of both tendencies. But cancer is recessive to non-cancer, and so *the first hybrid generation shows none of it, and in all my experience never has shown it,* but the tendency to cancer segregates out, and in the second hybrid generation it appears again, *in the same organs and in the same tissues of those organs which show the ancestral tumors.*

Note chart 1, showing three lines derived from strain 84, branch II. This chart is perfectly typical. The parent female 3931 died of carcinoma of the mammary gland, carcinoma of the lung, and pseudoleukemia. She was *hybridized* with absolutely non-cancer male 1364 who died of pulmonary infection. In accordance with the Mendelian expectation from such a cross, no cancer appears in the first hybrid generation (cancer is recessive to non-cancer). For the parents of this branch of the family female 6201 and male 4345, both heterozygotes, were selected.

Note how there was extracted from this hybrid cross three lines of mice: (A) the dominant, which neither in direct descent nor in any accessory fraternities, ever showed one case of neoplasm, malignant or benign; (B) the recessive line, 100 per cent malignant disease, and (C) the heterozygous line showing both cancerous and non-cancerous individuals. Note how the same types and locations of neoplasms which were bred into the strain with parent female 3931, segregate out and are transmitted as such wherever tumor occurs, both in the recessive, 100 per cent cancer line B, and in the heterozygous line C, namely carcinoma of the mammary gland, carcinoma of the lung, pseudoleukemia, and its closely related tumor type, thymus lymphoma.

It is interesting to note that in the animals of this stock, chronic leukemia and pseudoleukemia (alymphatic leukemia, not

STRAIN 84 - BR. II



SHOWING THE SEGREGATING OUT OF THE NON-CANCEROUS AND THE CANCER TENDENCIES, AND THEIR PERFECTLY TYPICAL MENDELIAN BEHAVIOR IN HEREDITY

CHART 1

lymphogranulomatosis) have occurred only in the cancer strains and have behaved as if they were true neoplastic diseases. As yet this material has not been fully analyzed and therefore the matter cannot at this time be discussed farther than the mere statement of the fact, which is in support of the contention of many pathologists, that chronic leukemia and pseudoleukemia are as much true neoplasms as are lymphosarcomas.

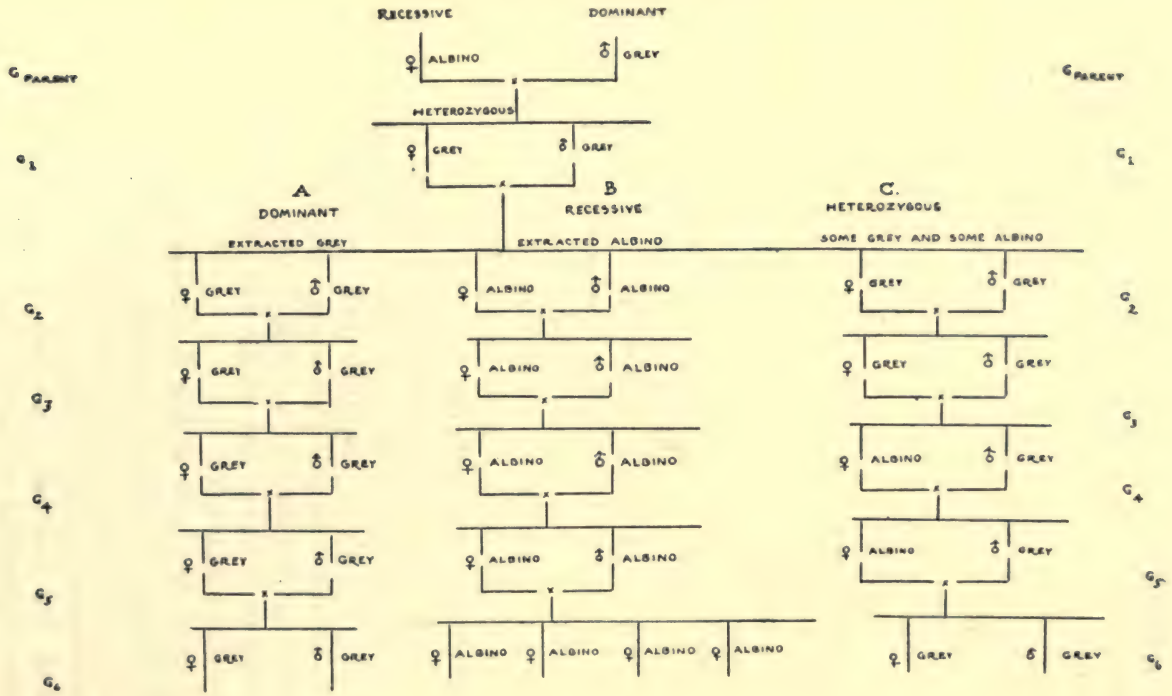
Chart 1, then, shows the segregating out and the transmission as such of the non-cancer and the cancer tendencies, also the tendency to a specificity of tissue type (which locates the neoplasm in a certain organ), and their perfectly typical Mendelian behavior in heredity as unit characters.

Note how exactly this follows the Mendelian expectation, as shown in chart 2, giving the classic behavior where a hybrid cross is made between the recessive albinism and the dominant pigmentation. Here also three lines are extracted, individual for individual parallel with those shown in chart 1: namely, first, a dominant line A, in which albinos never occurred either in the direct descent or in the accessory fraternities; second, a recessive line B, 100 per cent albinos, in which no pigmented individual ever appeared; and third, a heterozygous line C, showing some albinos and some pigmented mice.

Chart 2, then, shows the segregating out and the transmission as such, of the pigment making tendency and the lack of the pigment making tendency, and their perfectly typical Mendelian behavior in heredity as unit characters. That is, the pigment making tendency and the non pigment-making tendency behave in the matter of heredity just as did the cancer and the non-cancer tendencies in chart 1.

Chart 3 continues part of line A extracted from strain 84, branch II, *through the fifteenth generation without the occurrence of a neoplasm of any sort.* This shows the absolute segregating out and transmission as such of the non-cancer tendency. When a non-cancer line has once been extracted, spontaneous neoplasms never throughout my entire experience have occurred in such a strain again, unless cancer has again been hybridized in from an outside source. Never in line A nor in any of the accessory fraternities has a neoplasm of any sort whatever occurred.

MENDELIAN EXPLANATION CHART



SHOWING THE SEGREGATING OUT OF THE PIGMENTATION AND THE NON-PIGMENTATION TENDENCIES AND THEIR PERFECTLY TYPICAL MENDELIAN BEHAVIOUR IN HEREDITY

CHART 2

STRAIN 84 – BR.A

CONTINUED THROUGH THE 15 TH GENERATION

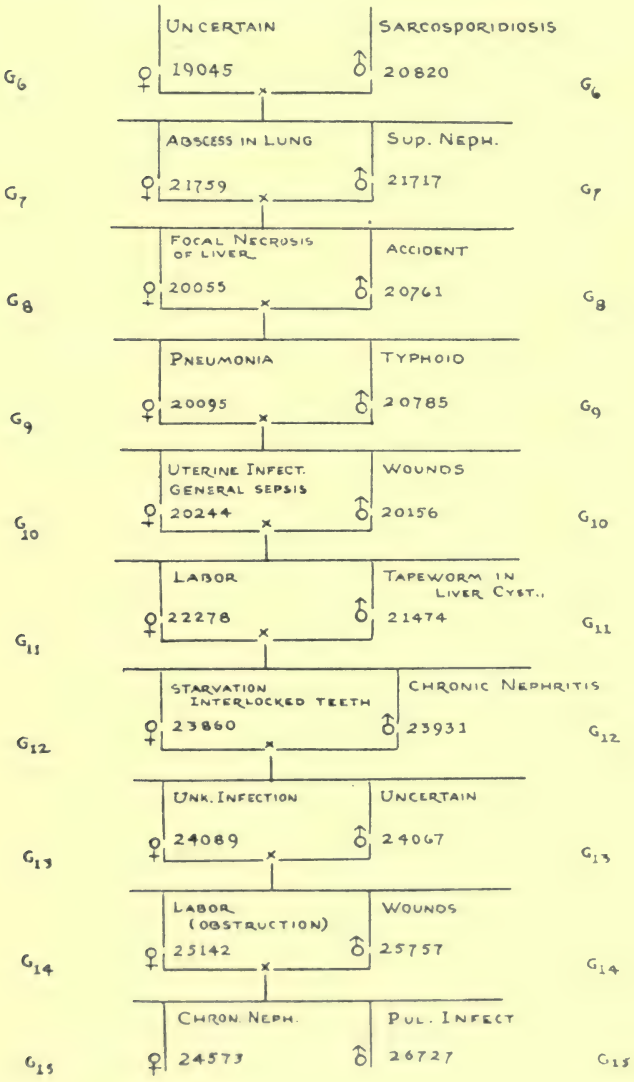


CHART 3

Chart 4 continues strain 84, branch II, line B, through the ninth generation. This is the 100 per cent cancer line extracted from this same cross. Note how the types and locations of the neoplasms occurring in this line of the strain are the same as

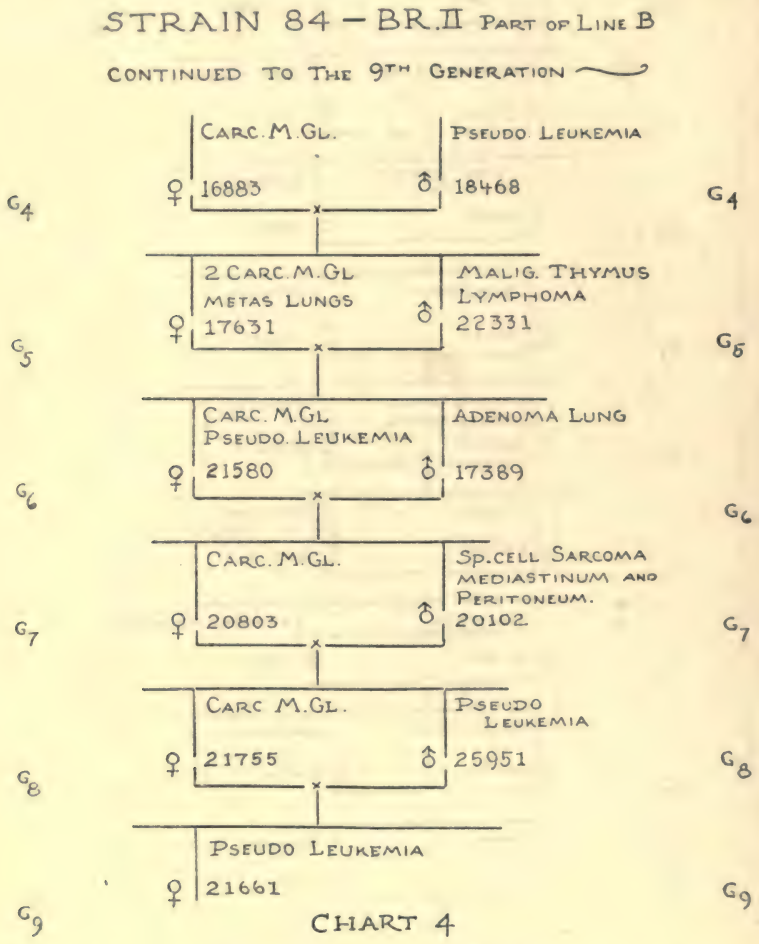


CHART 4

those bred into it in the original cross from female 3931, namely, carcinoma of the mammary gland, carcinoma of the lung, and pseudoleukemia, with its closely related tumor type, thymus lymphoma. Note female 21580 (generation 6) with the carci-

noma of the mammary gland of her grandmother and the pseudoleukemia of her grandfather. Note also the interesting sequence here of pseudoleukemia and thymus tumors through six consecutive generations, following the selection of male 18468 as the parent male in generation 4. Note also that the original parent female 3931 (shown in chart 1) had pseudoleukemia along with carcinoma of the mammary gland and primary carcinoma of the lung. Note male 20102 in generation 6, with a spindle cell sarcoma of the entire mediastinum and of the peritoneum. This single case of sarcoma was derived from an ancestor several generations antecedent to female 3931, the parent of strain 84. Lack of space prevents the showing of this ancestral sequence in the single chart.

Chart 5 continues line C of this same strain through the fourteenth generation. This is the heterozygous line. Note how, by the continued selective breeding of a heterozygous individual with a non-cancerous mate (as indicated by *H* and *N.T.* in the chart) all occurrence of neoplasms was held off until the thirteenth generation. Here, by the mating of two mice heterozygous to lung tumor, lung tumor occurred in the thirteenth generation, the parents concerned being female 22781 and male 22986. Lung tumor is one of the tumor types carried by this line. *By the right selective breeding in any heterozygous line, neoplasms can be made to occur or can be held off at will.*

Chart 6 shows part of strain 53, line A branching into two extracted 100 per cent cancer families, and line B, from the same parents, developing into a 100 per cent non-cancerous family. The heterozygous line is not shown in this chart, for lack of space. Note how, in line A family 1, a 100 per cent lung adenoma family is being extracted by the selection as parents of the family of two mice with lung adenoma, namely male 5334 and female 6490; while in family II of the same line, liver adenomas and mesotheliomas of the testicle are the prevailing tumors, there being four liver adenomas in the ten individuals forming the direct descent of these five generations.

Note that in line B after the first hybrid generation (female 5303 with a squamous cell carcinoma of the mammary gland)

STRAIN 84 – BR.C

CONTINUED THROUGH THE 14TH GENERATION

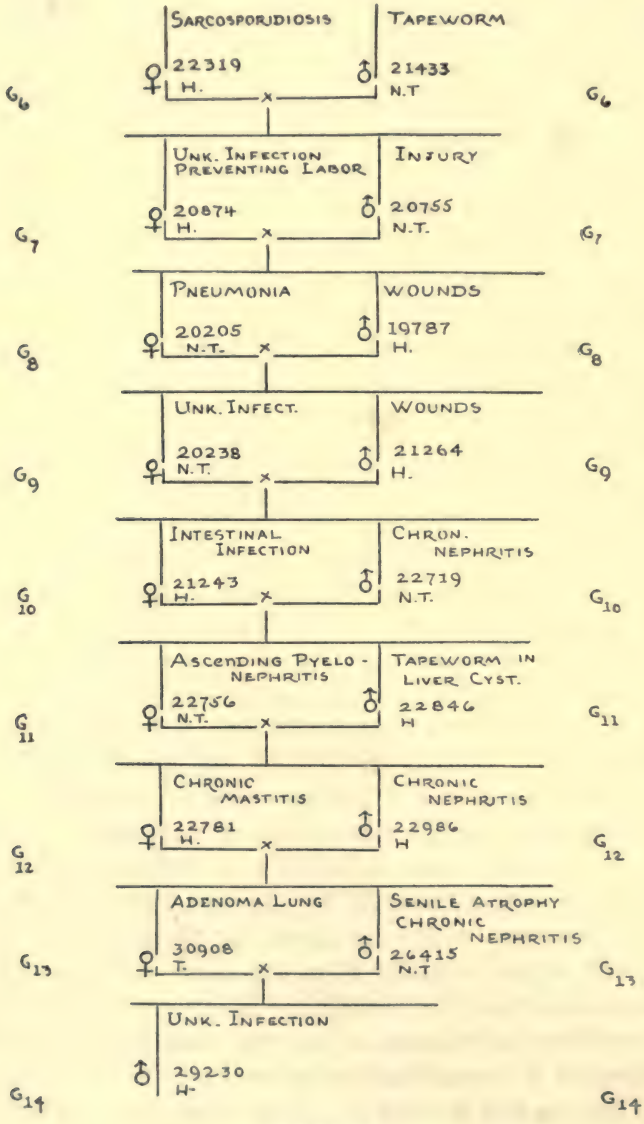


CHART 5

PART OF STRAIN 53

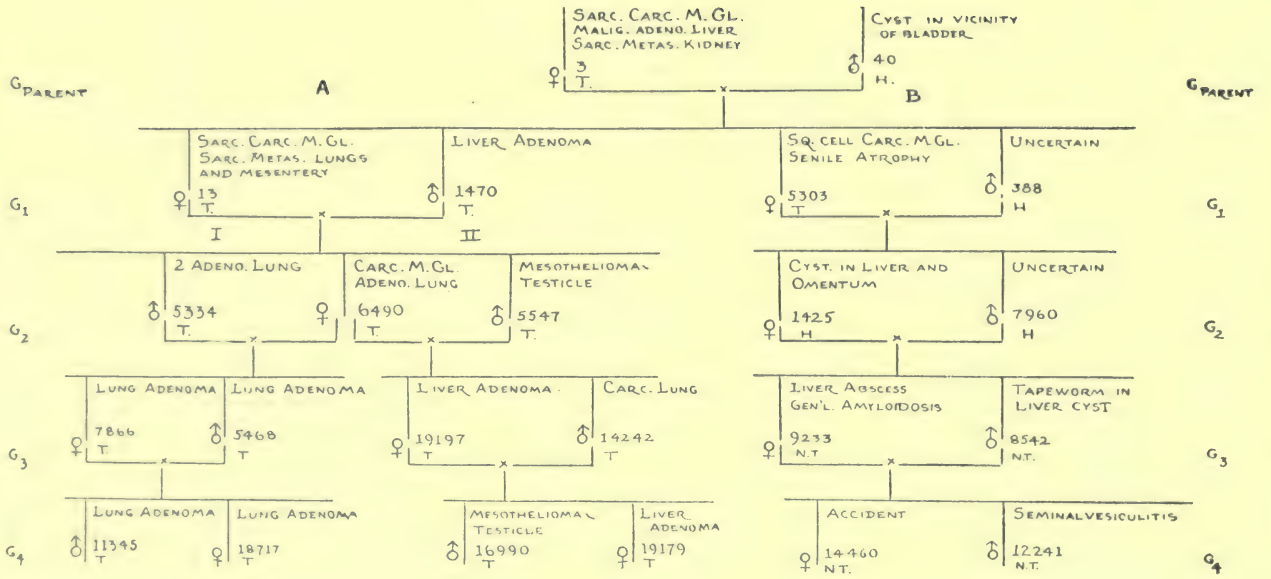


CHART 6

there was no further appearance of tumor. By the continued selection of non-tumorous individuals after the second filial generation, all neoplasms were completely ruled out of this branch of the strain.

Note female 13 of filial generation 1, line A, with a sarcoma-carcinoma of the mammary gland like her mother, female 3, and with secondary sarcomas in the lungs and mesentery. Note how, with her secondary lung tumor she was able to start a 100 per cent lung tumor line, the secondary lung tumor being as efficient as a primary lung tumor in transmitting lung tumor potentiality. This point is discussed at length in a previous report (16).

Note the prevalence of cyst and abscess formation in line B of strain 53, although in no case do these cysts or abscesses lead to tumor formation in this family. The offspring is made of the identical material of the germ plasm of its ancestry, and its tissues behave in the same way.

Chart 7 shows line B of this strain *continued through the eleventh generation without the occurrence of neoplasms.* Never, either in the direct descent nor in any accessory fraternity, did a neoplasm of any kind occur in this branch of strain 53 after the neoplastic tendency had once been bred out from the second filial generation.

Chart 8 shows part of strain 215 and some derivatives. Note the 100 per cent lung tumor strain being extracted in line A from female 5 with secondary carcinoma of the lung. Note the 50 per cent liver adenoma strain being extracted in line B. Strain 215 was made by the mating of female 3, who had a sarcoma-carcinoma of the mammary gland, a malignant adenoma of the liver, and sarcoma metastasis in the kidney, with male 360, who was proved heterozygous to lung and mediastinal tumors. Note how the different types and locations of neoplasms introduced by these two parents segregate out and are transmitted as such in the succeeding strain.

Charts 9 and 10 show part of strain 338, branch V with partial ancestry, and its offspring carried through the ninth generation. The original ancestor of this strain also was female 3, already referred to in chart 8. She had a sarcoma-carcinoma of the

STRAIN 53 - LINE B

CONTINUED THROUGH 11 TH GENERATION

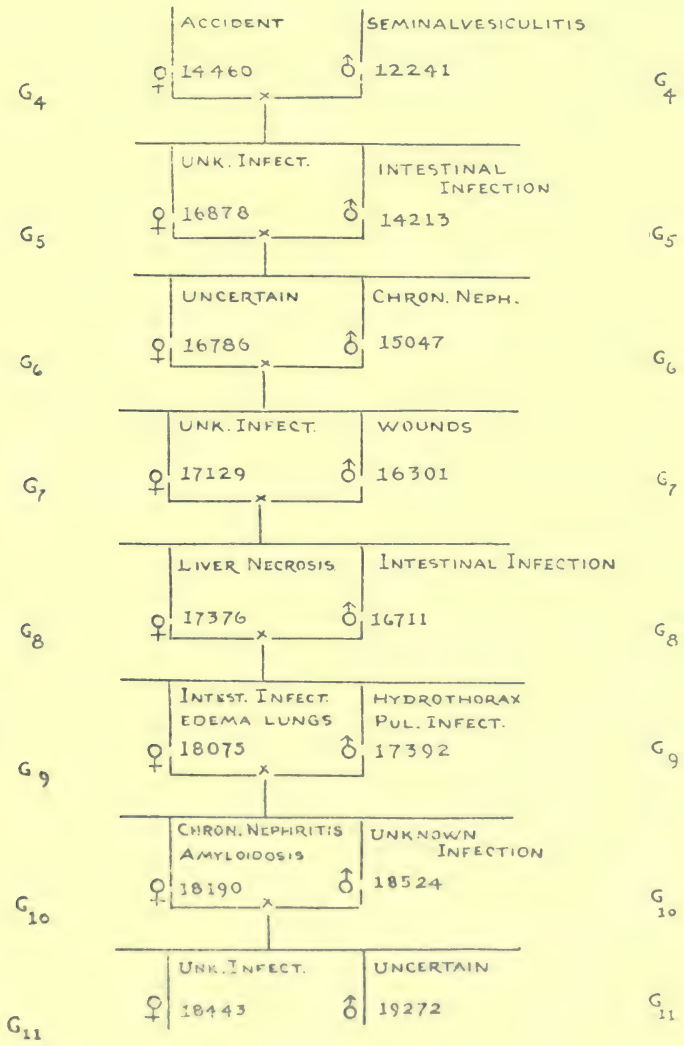


CHART 7

PART OF STRAIN 215 AND SOME DERIVATIVES.

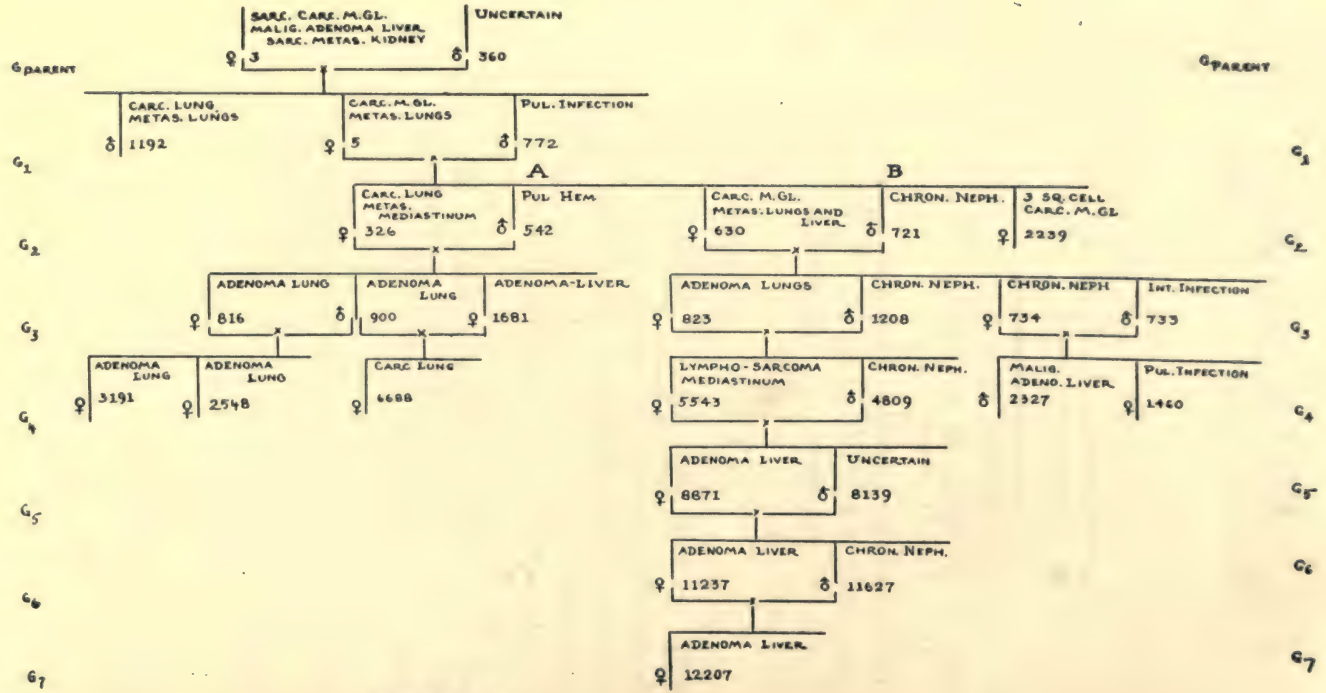


CHART 8

PART OF STRAIN 338 — BR. ♀ A — WITH PART OF ANCESTRY

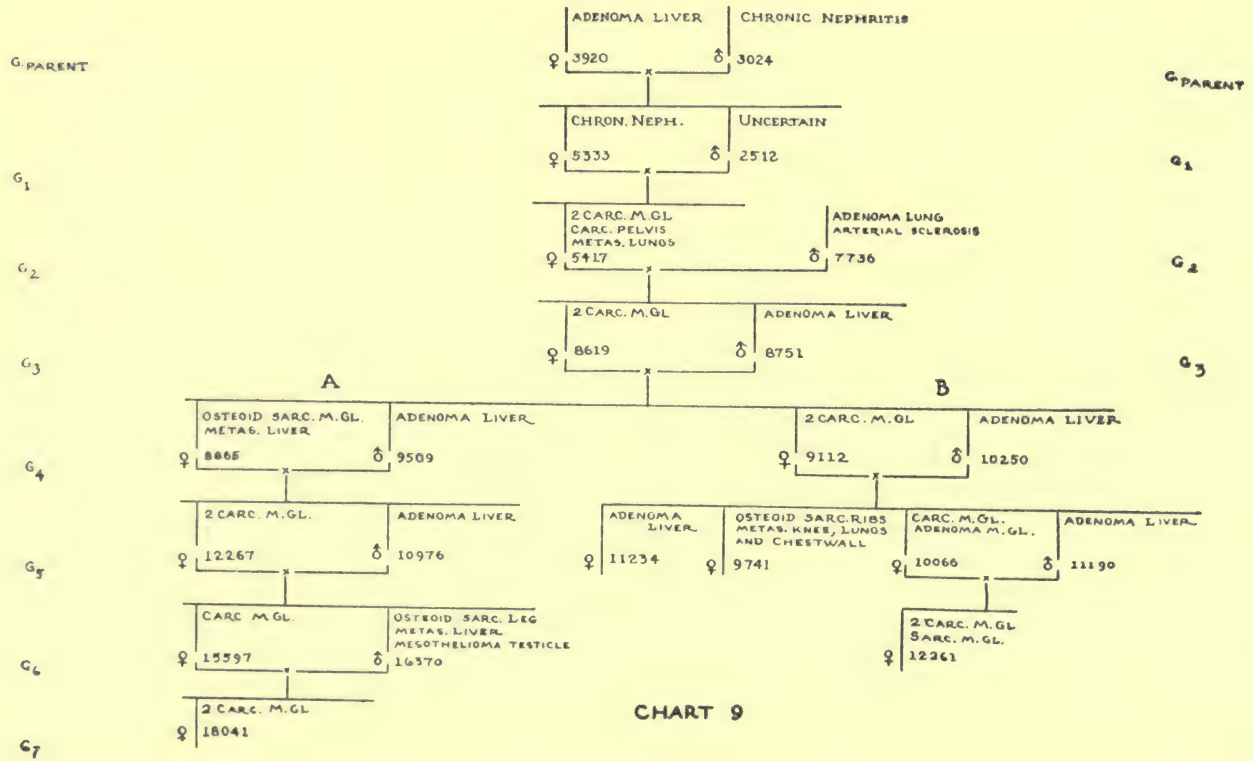


CHART 9

mammary gland, a malignant adenoma of the liver, and sarcoma metastasis in the kidney. Note the striking outcropping of liver tumors in this strain. Note how the different unit characters, sarcoma, carcinoma, and specificity of liver tissue type, get into

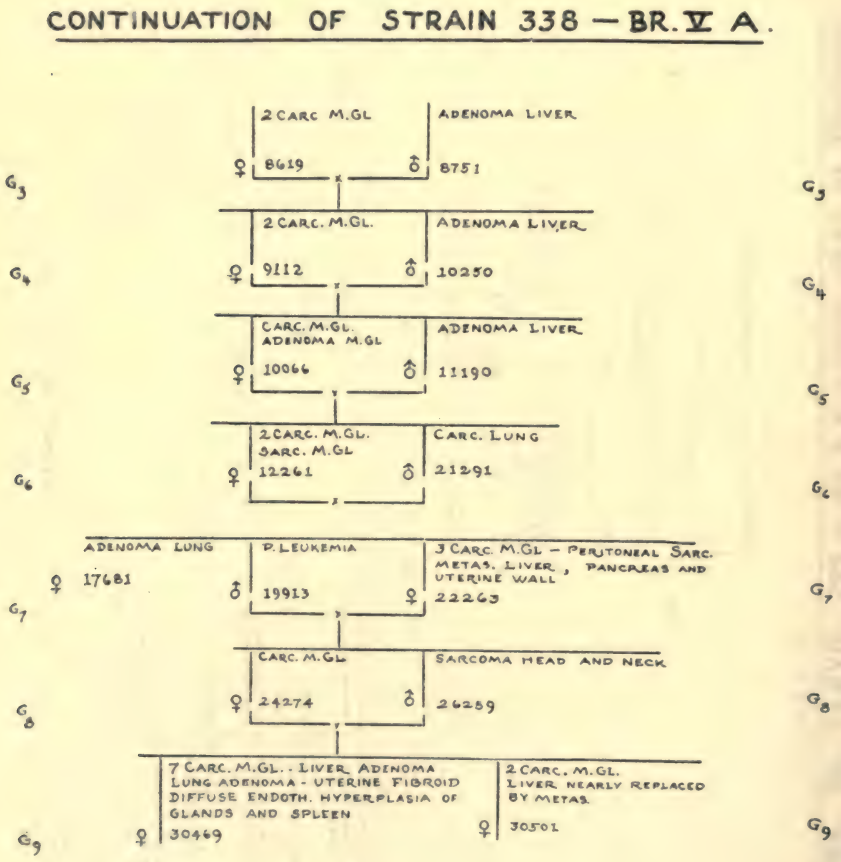


CHART 10

all possible combinations in the strain, so that even in the small number of individuals represented in these two charts, we find carcinoma of the liver, sarcoma of the liver, both primary and secondary, and adenoma of the liver.

That is, the carcinoma tendency segregates out and is transmitted as such. The sarcoma tendency segregates out and is transmitted as such. The adenoma tendency segregates out and is transmitted as such. A specificity of liver tissue which will insure its yielding to neoplasms, segregates out and is transmitted as such. These are all unit characters, and they get into all possible combinations even in this one small family alone.

I have from necessity selected only a few strains for the charts in this report. They are perfectly typical, however, and are representative of the hundreds of other strains, tumorous and non-tumorous, inbred and hybridized, which have been produced by selective breeding alone, and which have been analyzed in this laboratory for the past fifteen years. I have therefore been able to express the matter in little more than outline, and there are many and diverse ramifications of the work which, for the sake of unity, I cannot enter into at all at this time. But certain facts have consistently obtained in this work for twelve years, and these I wish to emphasize with their application.

Cancer and non-cancer have behaved consistently just as true albinism and pigmentation do in heredity. That is, just as true albinism is the total absence of the pigment-making mechanism present in the pigmented mouse, so *spontaneous cancer consistently behaves like the absence of a mechanism fitted to control proliferation and differentiation in regenerative processes.* At any rate, whether or not it is exactly this, it seems to be the absence of some controlling mechanism, and an animal either has it or has not, whether he is a mouse or a man.

Whenever spontaneous cancer comes out in a strain, it is because it has been bred in, in some degree; and however remote the cancer ancestry, we shall find it if we analyze far enough. *Moreover, we shall find not only the neoplastic ancestor, but the ancestors that carried the same types and the same locations of neoplasms shown in the later generations.*

There is no appearance of spontaneous cancer in any non-tumor strain. There are all percentages of cancer, some even as low as 0.1 in varying cancer strains. That is, cancer and non-cancer behave respectively like the absence and presence of a

particular controlling mechanism and an individual, whether it is a mouse or a man, either has it or lacks it.

The unit characters concerned in the heredity of spontaneous tumors, whether we are dealing with mice, with rats, or with man, are these: First, specificity of tissue type from organ to organ, which determines that the liver, or the kidney, or the uterus, or the mammary gland tissue, and so forth, shall be like the tissue of its ancestral organ (from which it was derived) and shall react in the same way to the same cause. For example, liver tumor begets liver tumor, etc. Second, a specificity of epithelium from ancestor to offspring which shall cause it to proliferate without differentiation and without control under a given provocation, i.e., carcinoma begets carcinoma. Third, a specificity of connective tissue from ancestor to offspring, which shall cause it to proliferate without differentiation and without control under a given provocation, i.e., sarcoma begets sarcoma.

Like all other unit characters, the unit characters here enumerated may get into all possible combinations; and we therefore, when dealing with fundamentally and completely analyzed stocks, have such a result as I have shown from female 3. This female 3, with a sarcoma-carcinoma of the mammary gland, a malignant adenoma of the liver, and sarcoma metastasis in the kidney, is able to transmit to her posterity (and has so transmitted) all possible combinations of these unit characters, namely: carcinoma of the mammary gland, sarcoma of the mammary gland, sarcoma-carcinoma of the mammary gland, adenoma of the mammary gland; carcinoma of the liver, sarcoma of the liver, adenoma of the liver; carcinoma of the kidney, sarcoma of the kidney, adenoma of the kidney.

There are only two possible methods of studying the inheritability of any character whatever, cancer or anything else. These are: First, the long, painstaking, difficult analysis of stock in the laboratory, so that we obtain analyzed individuals whose hereditary potentialities are known quantities and can be manipulated as such. This is the method which has been pursued for fifteen years in this laboratory. And second, the so-called statistical method which has been in vogue in the study of the inheritability of cancer in man.

All human statistics of this nature are based upon two things both of which may be in error. Namely, the memory of the patient, and the diagnoses concerning his ancestry. Rarely, back of one generation, are the facts remembered or the diagnoses based upon autopsy. We have then no certain scientific material whatever to deal with in these statistics.

But where these statistics are right, as they frequently even by chance must be, a biologic reading of them would show that they also demonstrate the inheritability of cancer in man.

Note chart 11 showing part of Strain 164 with partial ancestry in the maternal side. *Female 1236 who died of a thrombosed auricle,*[2] *was the parent female* of strain 164. Her mother was tumorous (indicated by *T* in the chart) and died of a highly malignant carcinoma of the mammary gland with metastases in the lungs. Her father, male 242, died of uncertain causes. He did not himself have tumor but was proved heterozygous to tumor (indicated by *H* in the chart); that is, he inherited it from cancerous ancestry and transmitted it to his offspring although not himself having tumor.

The male parent of strain 164 was a pure-bred house mouse, and died of uncertain causes. He was a member of strain 358, in my hands many years without the occurrence of tumor of any sort, malignant or benign, in any of its fraternities. He himself was an analyzed, proved non-tumorous mouse (indicated by *N.T.* in the chart).

In the first hybrid generation from this cross (namely, between a heterozygote and a non-tumorous mouse) no tumor of any sort has ever occurred. Cancer is recessive to non-cancer. In this chart the only members of the first hybrid generation of strain 164 that are shown are two pairs, namely, female 4224 and male 6480, the parents of branch III of the strain; and female 4921 and male 5652, parents of branch I of the strain. Female 4224 and male 6480 of branch III were both proved heterozygous to cancer, while female 4921 and male 5652, parents of Branch I, were both proved non-tumorous mice.

[2] This is a condition that has occurred with some frequency in this laboratory.

Note that the progeny in branch I (with both parents non-tumorous) were all absolutely non-tumorous. Nowhere, either in the direct descent nor in any of the accessory fraternities in this branch of the strain, has there ever been a single occurrence of tumor of any sort, malignant or benign. This is the classic

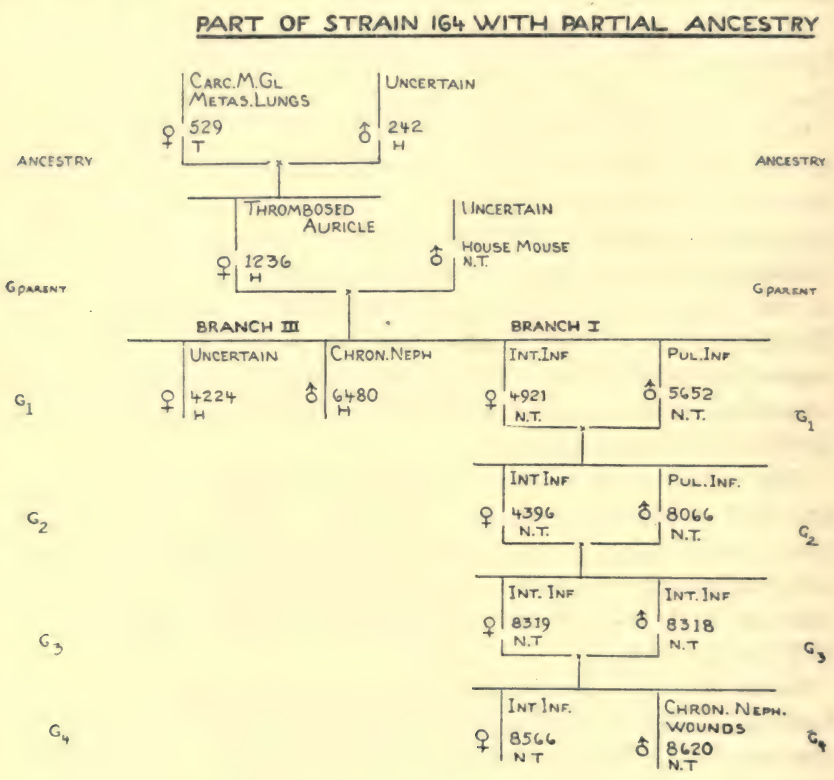


CHART 11

Mendelian behavior to be expected from the mating of two individuals carrying the dominant, that is, the non-cancer tendency, like female 4921 and male 5652. Branch I, then, strain 164, is an absolutely non-tumorous family, every member of which has been analyzed and has proved to be non-tumorous both in inbreeding and in hybridization, although this branch was

derived from cancer ancestry on the maternal side. This shows conclusively that the non-cancer tendency also segregates out and is transmitted as such, and that by the right selective mating the cancer tendency can be ruled out absolutely from a family, beginning with the second hybrid generation.

Chart 12 shows the origin of branch III of this same strain 164, and the parentage originating the three lines A, B, and C, in which this branch has been bred out. The succeeding charts will show the continuation of these three lines.

The parents of line A were tumorous male 3672 who died of a lymphosarcoma of the thymus, and heterozygous female 8419 who died of peritonitis. Their two offspring selected to carry on this line were both heterozygous to tumor, namely, female 10597 who died of tapeworm, and male 8521 who died of intestinal infection.

The parents of line B were non-tumorous female 7126 and heterozygous male 6728. Their offspring selected to carry on line B were heterozygous female 8146 and non-tumorous male 8150, both dying of intestinal infection.

The parents of line C were heterozygous female 7913 and tumorous male 8276 who died of sarcoma of the mammary gland. Their offspring selected to carry on line C were tumorous female 10537 with a carcinoma of the mammary gland, and non-tumorous male 8247 who died of chronic nephritis. This chart, then, shows how lines A, B, and C of branch III, strain 164, originated.

Chart 13 shows line A of branch III, strain 164, continued through eight generations. Note now, how by the right selective matings of heterozygous and non-tumorous mice, the occurrence of malignant disease is held off until the sixth generation. If, now, female 12876 with a lymphosarcoma of the mesentery, left kidney, and right ovary, had had her statistics taken in the hospital without error even for three generations, no statistics of tumor would have appeared; nevertheless the inheritance of her tumor type is direct from her grandfather four generations back. By the mating of two heterozygous individuals from this tumor mother, namely female 11551 and male 11440, again in the next

PART OF STRAIN 164-BRANCH III
SHOWING HOW LINES A, B AND C ORIGINATED

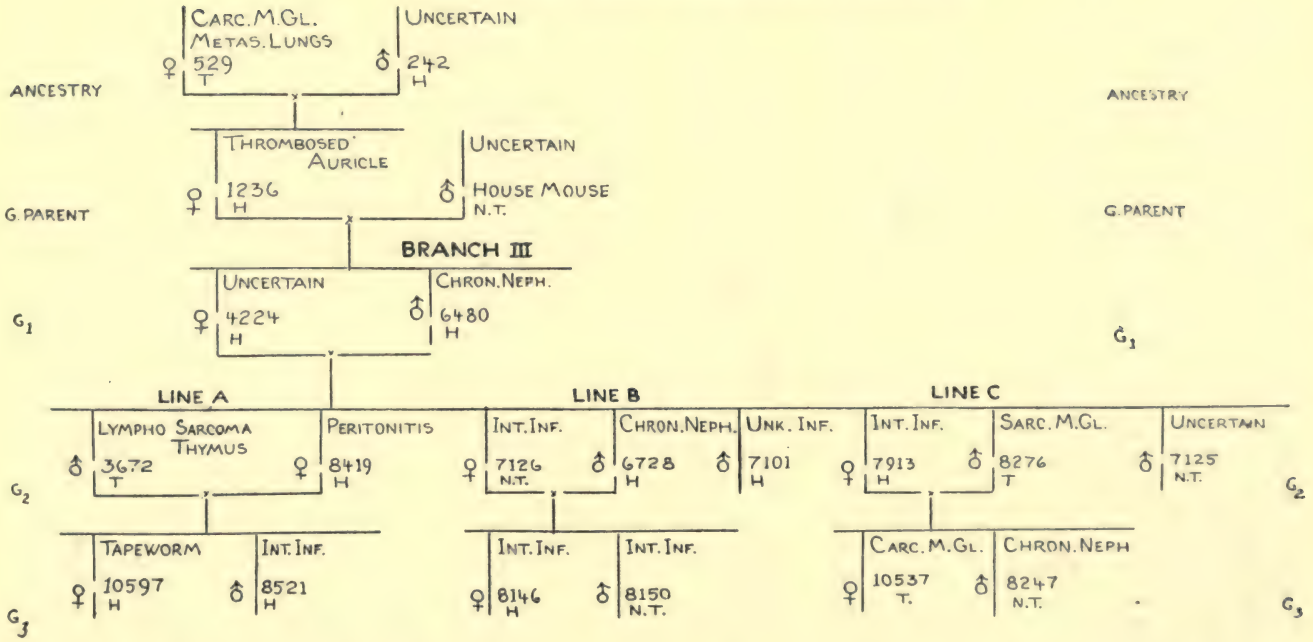


CHART 12

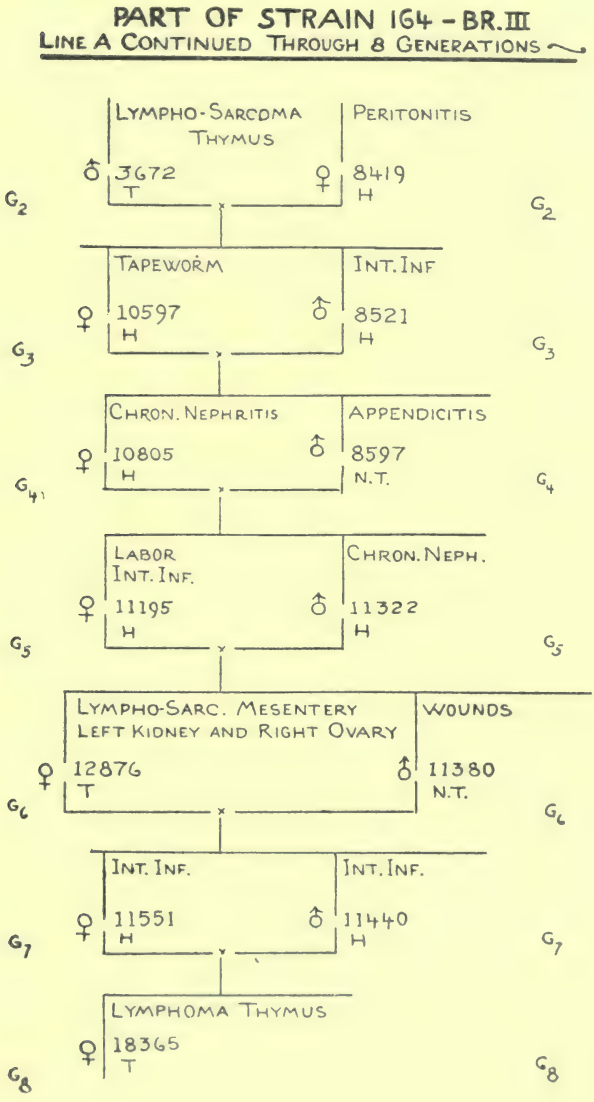

PART OF STRAIN 164 - BR.III
LINE A CONTINUED THROUGH 8 GENERATIONS
LYMPHO-SARCOMA THYMUS
3672 T
PERITONITIS
8419 H
G2
G2
TAPEWORM
10597 H
INT. INF
8521 H
G3
G3
CHRON. NEPHRITIS
10805 H
APPENDICITIS
8597 N.T.
G4
G4
LABOR INT. INF.
11195 H
CHRON. NEPH.
11322 H
G5
G5
LYMPHO-SARC. MESENTERY LEFT KIDNEY AND RIGHT OVARY
12876 T
WOUNDS
11380 N.T.
G6
G6
INT. INF.
11551 H
INT. INF.
11440 H
G7
G7
LYMPHOMA THYMUS
18365 T
G8
G8

CHART 13

generation a malignant thymus lymphoma occurs in female 18365. At the same time, all other types and locations of tumors are ruled out from this strain by the right selective matings.

Chart 14 shows line B of branch III, strain 164, continued through nine generations. Note how by continued matings of heterozygous and non-tumorous individuals, all occurrence of malignant disease has been held off until the eighth generation. If female 21189 of the eighth generation, who died of an osteosarcoma of the leg and spine, had had her hospital statistics taken even seven generations without error, there would have been no appearance of tumor. Nevertheless, by tracing far enough back, we find the ancestor with malignant disease. This chart shows with what certainty the tendency to neoplasms can be manipulated and controlled. Through generation after generation it was carried along by heterozygous individuals, certain to appear when the right matings were made.

Chart 15 shows line C of this branch of strain 164 continued through seven generations. Here by mating a tumorous female 10537 with a proved non-tumorous male 8247, no tumor occurred in the next generation. But by the selection of two heterozygotes of generation 4, carcinoma appeared in the succeeding generation (generation 5) in female 12779 with a squamous cell carcinoma of the neck and pseudoleukemia. Again, by the mating of this female with a heterozygous male 16667, note how both pseudoleukemia occurred in the next generation, male 14631, and also squamous cell carcinoma of the neck, which at death had extended anteriorly and obliterated the face (male 14583). Female 18413, daughter of male 14583, showed the carcinoma of the mammary gland of her grandmother four generations back and the sarcoma of her grandfather five generations back. When a given type or location of neoplasm is bred in, it can be manipulated with absolute accuracy by the type of selective breeding used. It can be made to hold off for any number of generations desired or to appear in the next generation, as in the case of male 14583 with a squamous cell carcinoma of the neck in generation 6, following female 12779 with a squamous cell carcinoma of the neck in generation 5; or male 14631

PART OF STRAIN 164 BR III
LINE B CONTINUED THROUGH 9 GENERATIONS

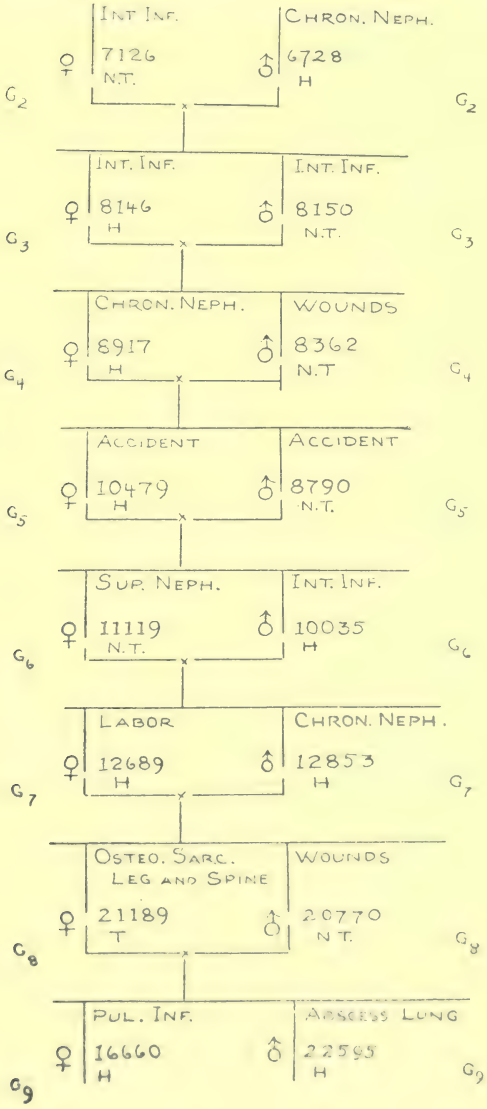


CHART 14

with pseudoleukemia in generation 6 following female 12779 with pseudoleukemia in generation 5.

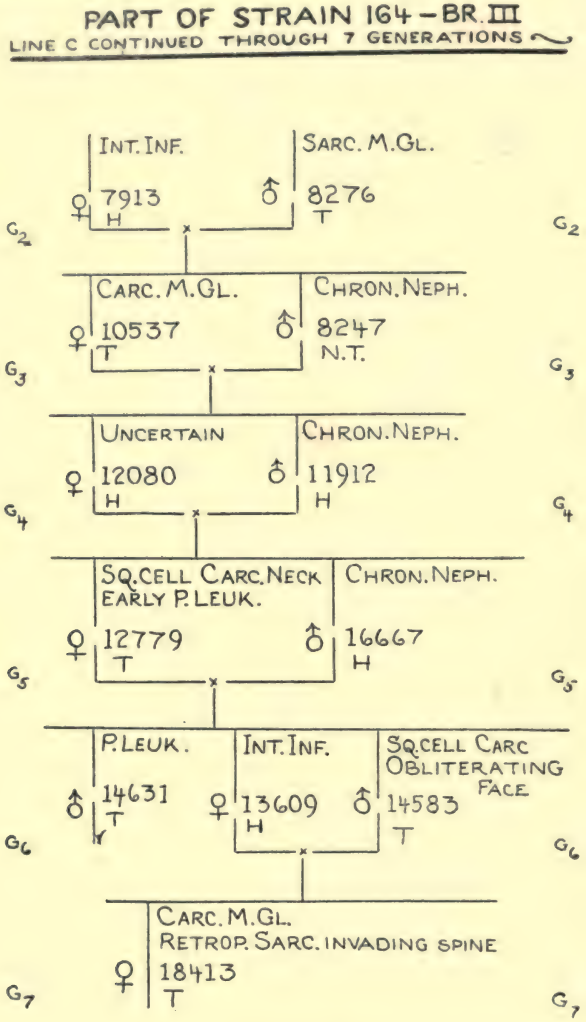


CHART 15

If human cancer statistics when correctly taken were biologically read, they would show as certainly as do mouse statistics the inheritability of cancer. They would show that the human

heterozygote carries and transmits neoplastic tendencies exactly as do mouse heterozygotes, although they themselves do not develop the disease. This follows exactly the classic Mendelian pattern from the mating of pigment-bearing with non-pigment-bearing mice.

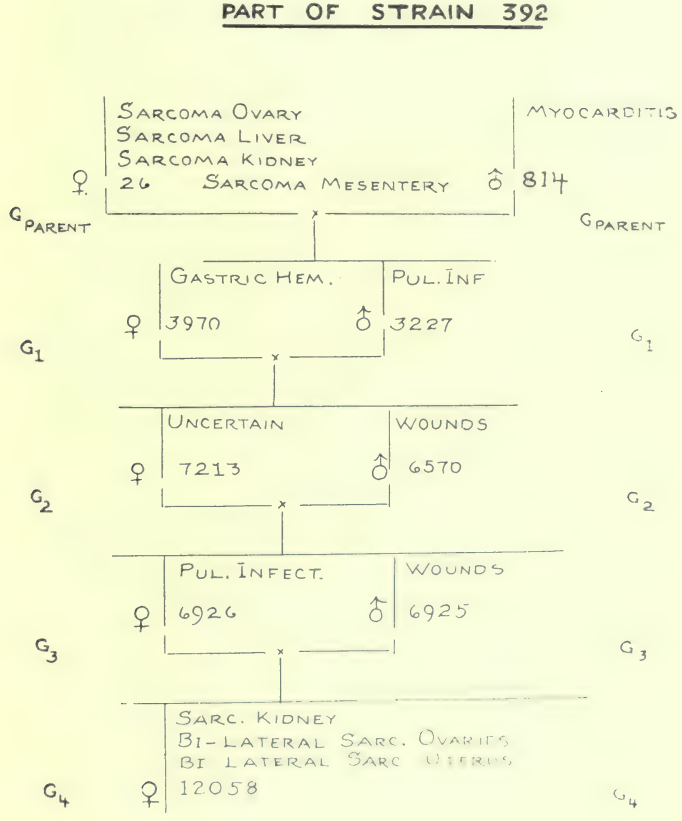


CHART 16

Chart 16 shows part of strain 392. The parent female 26 had a sarcoma of the ovary, sarcoma of the liver, sarcoma of the kidney and perirenal tissues, and a sarcoma of the mesentery. She was mated with male 814 who died of myocarditis. By the right selective mating, the occurrence of sarcoma (and all other types

of neoplasms) was held off through the three succeeding generations. In the third hybrid generation, by the selection of two individuals heterozygous to sarcoma, sarcoma appeared in the immediate offspring (generation 4), female 12058 repeating the sarcoma of the kidney and of the ovaries of her grandmother four generations back, adding also bilateral sarcoma of the uterus. No present day hospital statistics could have shown the correct causes of death through four generations. If, therefore, this had been a human case, there would have been no record of tumorous ancestry—yet there is here the most evident and perfect persistence and final emergence of the exact type and locations of neoplasms, through the right selective breeding to bring it out.

Again note chart 17, showing strain 465 with partial ancestry. Let me go through this chart somewhat in detail, in order that we may see the perfect evidence it affords of *first, the segregating out of tumor types and their consequent inheritability, and second, the segregating out and consequent inheritability of a specificity of organ tissue type, transmitted through generation after generation both where inbreeding and where hybridization was employed.*

Female 3 and male 30 were the ancestors of the paternal side of this strain. Female 3 had a sarcoma-carcinoma of the mammary gland, a malignant adenoma of the liver, and sarcoma metastasis in the kidney. Male 30 was proved heterozygous to tumor. This is a case, then, of mating a tumorous individual (recessive) with a heterozygote. In accordance with the Mendelian expection from such a cross, tumor comes out in the first hybrid generation; namely, female 883 with an adenoma of the liver.

In the first filial generation this female 883, with an adenoma of the liver, was *hybridized* with male 842 who died of uncertain causes, but who was proved heterozygous to tumor. Their son, male 1011, dying from acute nephritis, was heterozygous to liver tumor. He was *hybridized* with female 441 (entirely unrelated) who was also heterozygous to tumor. Their son, male 3024, was *hybridized* with female 3920, who came of a liver tumor ancestry and who herself had an adenoma of the liver. Note the outcropping of liver tumor in the second hybrid genera-

PART OF STRAIN 465–WITH PARTIAL ANCESTRY

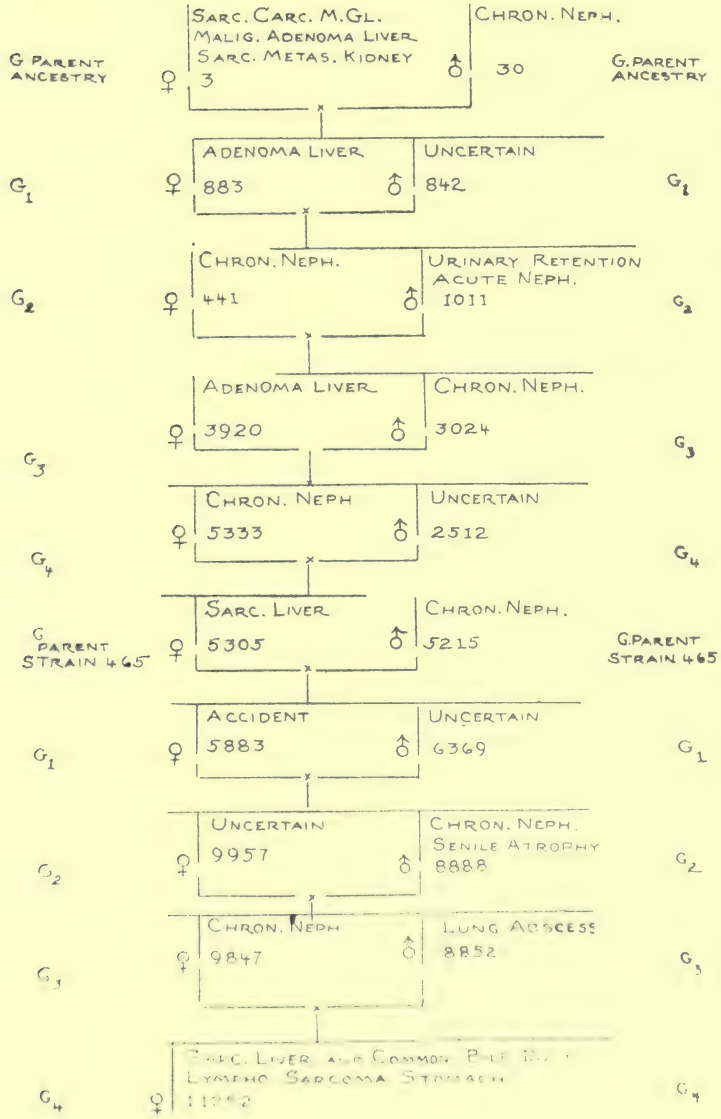


CHART 17

tion, female 5305 with a sarcoma of the liver. Note here how there segregated out on the one hand the unit character sarcoma introduced by female 3, five generations back, and on the other hand the specificity of liver tissue type locating tumors in the liver which also was originally introduced by female 3, transmitted through and reinforced by female 883 and female 3920. Note how in this generation the unit character sarcoma got into combination with liver tissue of a type to yield to neoplasmic growth (another unit character), so that we have in female 5305 a *sarcoma* of the *liver*.

This female 5305 was *hybridized* with male 5215, who died of chronic nephritis. Through three succeeding generations, by the right selective matings, all occurrence of neoplasms was held off. But the certainty of its transmission by heredity is indisputably demonstrated, for by the use of analyzed individuals two mice heterozygous to liver tumor were selected in the third filial generation, namely female 9847 who died of chronic nephritis, and male 8852 who died of a lung abscess; and liver tumor appeared in the next generation. Not only liver tumor occurred, but the same combination of unit characters occurred, *namely the combination of sarcoma, and the neoplastic tendency in the liver.*

Let me summarize the facts demonstrated in this chart (chart 17).

1. Sarcoma segregated out and was transmitted as such.
2. Adenoma segregated out and was transmitted as such.
3. Other types of neoplasms segregated out and were not transmitted at all in this line of succession.
4. A specific type of liver tissue (viz., lacking the non-cancer mechanism) segregated out and was transmitted as such, so that five liver tumors occurred in this small family alone.
5. A specific type of tissue in all other organs (viz., possessing the non-cancer mechanism) segregated out and was transmitted as such, so that all other organs refused neoplastic growth, with the single exception of female 11252, whose liver sarcoma spread by extension into the common bile duct, and who had also a lymphosarcoma of the stomach; but it is notable that this is the family in which nearly all of the few stomach tumors in this stock have occurred.

In the light of such perfect evidence as this, it is logically absurd to question the segregating out as unit characters of the sarcoma tendency, the carcinoma tendency, adenoma tendency, and the tendency to a specific type of organ tissue determining the location of neoplasms, and their transmission as such by heredity. Moreover, when we have such analyzed human individuals and such exact data concerning their neoplasms (if such a time ever comes) *we shall find that exactly the same laws govern the transmisson and occurrence of human neoplasms, otherwise there is no such thing as biologic or organic law.*

Charts 11 to 17 inclusive show how the tendency to neoplasms of specific types and of specific organs is carried along by heterozygotes, and how by the right selective matings alone, both in inbreeding and in hybridization, neoplasms of these types and of these organs can be held off or brought out at will in the resulting strains by the use of analyzed individuals. Such neoplastic tendencies can be carried along by heterozygous individuals through any number of generations desired, just as the tendency to albinism can be carried along by heterozygotes as long as may be desired. But by right selective breeding, both the neoplastic tendency and the albinic tendency can be made to emerge at will.

The heterozygote, then, the product of hybridization in any species or any variety, in whom the recessive (cancer or albinism etc.) lies hidden but potent for transmission, may be a very puzzling factor in heredity; for he contains in his germ plasm, and therefore can transmit to his offspring, unit characters different and frequently opposite in nature. As, for example, a pair of heterozygous black mice transmitting albinism to their immediate offspring, or a pair of heterozygous non-cancer mice transmitting cancer to their immediate offspring, because potential cancer went into the germ plasm from which the heterozygotes developed. The heterozygotes in the human cancer problem have been the individuals who have blinded the readers of human statistics to the fact of the inheritability of human cancer, hiding as they do the recessive (cancer) behind the dominant appearance, which is appearance only.

There is a very widespread objection in the medical profession today to the thought that cancer is inheritable, and a very widespread and ready categorical denial of its inheritability, on the basis of these erroneous and misread human cancer statistics. As the opinion of many physicians and surgeons in this matter is based upon the statements made by the Society for the Control of Cancer, let me here quote from this year's annual leaflet put out by the Society, item no. 6: "Cancer is not inherited. It is not certain even that a tendency to the disease is inherited."

Let us subject this excerpt to the biologic test. I have reminded you that man repeats in his embryonal development the history of organic evolution. He begnis as a single cell. In this cell there is no nose, no legs, no vertebrae arranged in a perfect and beautiful spinal column, no liver, no epithelium, no cancer. What resides in this single cell is the tendency to all these things. That is the basis of all heredity. All inherited characters are inherited tendencies of the cell. There is no other form of inheritance.

I have emphasized the evolutionary basis of the law of heredity, a common law of protoplasmic behavior—What goes into the germ plasm comes out in the offspring; the fundamental necessity of similar tissues behaving in similar fashion if there is to be such a thing as species or race. The mouse tumors under study in this laboratory are spontaneous tumors, arising in the natural life of the animal without artificial interference of any sort except that of selective breeding, exactly as man's tumors arise. They arise in the same tissues and in the same organs as the tumors of man; they follow the same clinical course; they cause death in the same ways. Under the microscope they present the same appearance as similar tumors in similar organs in man. *They are the same biologic entity as similar tumors in similar organs in man. And consequently, if we do not discard the theory of evolution, we must admit that they behave in the same way in the matter of heredity as in all other matters.*

When we have found, as we have found in this laboratory for twelve years, that carcinoma and non-carcinoma tendencies segregate out and are transmitted as such; that sarcoma and

non-sarcoma tendencies segregate out and are transmitted as such; that a specificity of tissue type in specific organs segregates out and is transmitted as such, so that an organ either has or lacks the non-cancer mechanism; *when, I say, we have found that these unit characters segregate out and are transmitted as such in mice, so that we can analyze individuals and manipulate these unit characters in heredity as you can pour HCl on Zn with a known outcome—unless we discard the entire biologic science of today, we must admit that these same unit characters segregate out and are transmitted as such in man.*

Moreover, the human statistical evidence admitted by the most vigorous opponent of cancer heredity, itself demonstrates the inheritability of cancer in man, when it is correctly and biologically read.

SUMMARY

1. Cancer and non-cancer tendencies segregate out and are transmitted as such.

2. They are therefore unit characters.

3. A specificity of tissue type in specific organs from ancestor to offspring segregates out and is transmitted as such.

4. It is therefore a unit character.

5. Since these things are unit characters, it is possible to manipulate them by selective breeding and thereby to implant them indelibly in any species, *or to eliminate them permanently and completely from any species.*

6. Cancer and non-cancer behave like the absence and presence respectively of a mechanism fitted to control proliferation and differentiation in regenerative processes, and an animal either has this mechanism or lacks it, no matter to what species he may belong.

7. There is therefore a ready and certain genetic method of escape from cancer for the individual and for the race.

8. The demonstration of the inheritability of cancer and non-cancer tendencies in mice is a demonstration of the inheritability of these tendencies in man and in all other species which show cancer, if we are to maintain the theory of evolution and to admit that there is such a thing as biologic law.

9. The study of cancer behavior, which has demonstrated itself to be fundamentally a biologic problem, makes evident the necessity of understanding and considering the biologic facts underlying all pathologic conditions.

10. And, therefore, when we have got biology under all our pathology and bacteriology, all our physiology and therapy, there will no longer be these monstrous diseases, but only the slow and natural death which is the fatigue and diminution and final cessation of the organ and the organism.

11. From the procedure of analyzing stock into its unit characters in order to manipulate the cancer tendency, there has emerged the fundamental law of heredity—What goes into the germ plasm must come out in the offspring.

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(3) Slye: The incidence and inheritability of spontaneous cancer in mice. Third report. Jour. Med. Res., 1915, n. s., xxvii, 159.

(4) Slye, Holmes, and Wells: Primary spontaneous tumors of the lungs in mice. Fourth report. Jour. Med. Res., 1914, n. s., xxv, 417.

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(17) Ewing: Neoplastic Diseases, 2d edition, New York, 1922, p. 105.

The Function of the Colostrum

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THE FUNCTION OF THE COLOSTRUM*

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Recent studies on the chemistry of milk proteins and on their behavior in immunologic reactions [1] have incidentally brought into the foreground certain hitherto insufficiently appreciated facts concerning the colostrum, which seem to have much practical significance. The production by the mammary glands for a few hours after parturition of a secretion, colostrum, which is quite different in composition from the true milk that is thereafter their chief secretion, seems to be a universal occurrence in all mammals, and hence cannot well be a fortuitous circumstance without significance; and yet the textbooks on obstetrics and pediatrics do not indicate that any considerable importance is generally attributed to this secretion.

Analysis of bovine colostrum by Crowther and Raistrick [2] disclosed that the colostrum from early milkings contains on an average as much as four times more protein than the milk of the same cow, this increase being due to the presence of a great quantity of globulin, added to about the usual quantity of milk albumin and casein. Within forty-eight hours after lactation, the globulin content falls to about one-tenth the quantity that was present in the first colostrum. These authors found that from the chemical standpoint casein, lactoglobulin and lactalbumin are sharply differentiated and distinct proteins, and that they have the same composition whether prepared from colostrum or

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1. These are fully reviewed by Wells, H. G., and Osborne, T. B.: Anaphylaxis Reactions with Proteins from Milk, J. Infect. Dis. **29**: 200 (Aug.) 1921.

2. Crowther, Charles, and Raistrick, Harold: A Comparative Study of the Proteins of the Colostrum and Milk of the Cow and Their Relations to Serum Proteins, Biochem. J. **10**: 438, 1916.

from milk. The lactoglobulin resembles in chemical composition the globulin of ox blood serum, but lactalbumin is quite distinct chemically from serum albumin, and casein is entirely different from any known blood protein. Applying the racemization method of Dakin to the study of the character of milk proteins, Woodman [3] found that even in the fine details of structure of protein molecules which this method brings out, the identity of the globulins from cow's milk, colostrum and serum was demonstrated; also that the albumin of colostrum and milk are the same, but distinctly different from the serum albumin.

The immunologic studies of Wells and Osborne, corroborating and extending the results of earlier workers in this field, established that the globulin of milk behaves in its biologic reactions exactly the same as serum globulin, but that the other three known proteins of milk (casein, lactalbumin and alcohol-soluble protein) have no biologic counterparts in the serum.

These mutually confirmatory observations, together with many similar ones made previously, all establish that colostrum is a fluid that is characterized by consisting of an extremely rich solution of globulin (about 8 per cent.) added to the usual proportions of casein and albumin of milk, and that this globulin is identical with serum globulin, being the only protein of milk that is identical with any of the blood proteins. What is the purpose of this great secretion of globulin into the milk for a few hours after the young are born? It evidently is a true secretion, and not any mere leakage of proteins from the vessels, since the serum albumin and fibrinogen do not appear in the colostrum, and the globulin content of colostrum may be much higher than that of the blood. Undoubtedly it is related to the fact that the serum globulin fraction contains or carries the antibodies of the blood,[4] and that numerous investigations on the defensive mechanism of new-born animals have shown that the suckling animal secures important accessions to its antibody defense during the first days of life. It is generally recognized that young

3. Woodman, H. E.: A Comparative Investigation of the Corresponding Proteins of Cow and Ox Serum, Cow's Colostrum and Cow's Milk by the Method of Protein Racemization, Biochem. J. **15**: 187, 1921.

4. It has not yet been determined whether the antibodies actually are globulins or not.

infants possess a greater or less degree of immunity to various infections. Often this early immunity is but transitory, which indicates that it is a passive immunity, and therefore to be attributed to antibodies obtained from the mother, whether through the placenta or through the colostrum.

The literature on this subject to 1912 has been carefully reviewed by Famulener,[5] who found it to show that when the mother exhibits a high degree of immunity at the time of birth of young there is some passive transfer of immunity, either through the placenta before birth or by means of the milk immediately after birth. Famulener immunized goats to sheep erythrocytes during gestation, and found that the blood of the newly born kids taken before they had suckled showed no appreciable amount of hemolysin, so that evidently little placental transfer of this antibody had taken place. The colostrum of the immunized mothers was found rich in hemolysins, often much richer than the serum of the same animal, and kids getting the colostrum quickly acquired a high antibody content in the blood. The milk soon lost its hemolysin content after the colostrum stage, and lactating goats immunized after gestation did not secrete antibodies in their milk in quantities comparable to that found in the colostrum, and often the milk contained no demonstrable antibodies. Older sucklings did not absorb antibodies from the milk to such an extent that they could be demonstrated in the blood. It is to be recalled that early investigators found that the newly born mammal is able to absorb proteins from the alimentary canal in an unchanged condition to a much greater extent than later, when the digestive processes have become more developed.

If one compares the curve of antibody titer in the colostrum and milk observed by Famulener, it will be seen to correspond perfectly with the curve for globulin content established for colostrum and milk by Crowther and Raistrick; that is, the early colostrum may contain much more antibody and globulin than the mother's serum, and both fall in from twenty-four to forty-eight hours to a very low figure, to continue at a low,

5. Famulener, L. W.: The Transmission of Immunity from Mother to Offspring, J. Infect. Dis. **10**: 332, 1912.

often negligible level in the milk thereafter. Famulener expressed clearly the idea that the colostrum is characterized by containing large amounts of antibodies, probably associated with its high globulin content, and that these are absorbed unchanged from the alimentary canal into the blood of the new-born suckling animal, but the practical importance of his contention seems to have been generally overlooked. More recent developments in our knowledge of the chemistry of colostrum have served to support the correctness of his deductions, and such experimental work as has been done on the immunology of the new-born has corroborated his experimental results. Thus, Reymann [6] found that, at least in the case of agglutinins in goats, the young were usually born without agglutinin but acquired a considerable amount during the first days of life from the mother's milk. He found also that the colostrum is especially rich in these immune bodies, commonly being richer in agglutinins than even the serum of the same animal, just as the colostrum may be richer in globulin than the blood of the same animal. The same thing seems to be true of antibodies other than agglutinins. The amount of antibodies in the milk falls rapidly during the first two or three days of lactation, and usually the later milk does not contain quantities large enough to be demonstrated; in other words, the antibody content of the lacteal fluid parallels the globulin content. Also, Howell and Eby [7] found that the antibodies of the mother's blood diminish after parturition, presumably because they are being poured out into the colostrum.

Little and Orcutt [8] found that when the blood of cows contains specific agglutinins for *B. abortus,* through natural infection, these agglutinins are also present in the colostrum, and often the concentration is higher in the colostrum (three of four cases), but the blood of the calves when born contains no agglutinins; only after the calves have suckled the colostrum does

6. Reymann, G. C.: On the Transfer of the So-Called Normal Antibodies from Mother to Offspring, J. Immunol. **5**: 227, 1920.

7. Howell, Katherine M., and Eby, Harriet: The Transmission of Specific Immune Bodies from the Mother to the Young, J. Infect. Dis. **27**: 550 (Dec.) 1920.

8. Little, R. B., and Orcutt, Marion L.: The Transmission of Agglutinins of B. Abortus from Cow to Calf in the Colostrum, J. Exper. Med. **35**: 161, 1922.

their blood contain the agglutinins, and then the titer may rise to a higher level than that of the mother's blood. When the colostrum was withheld and milk of a low or negative agglutinin titer substituted, agglutinins failed to appear or to accumulate in the blood of the calf. Agglutinins begin to appear about an hour after the calf has ingested colostrum, and may reach a maximum within five hours after feeding. This work is of particular significance since it deals with immune bodies the results of natural infection, and, with the previously quoted work, seems to establish definitely

PROTEIN CONTENT OF HUMAN BLOOD

	Total Nitrogen	Euglobulin Nitrogen	Pseudoglobulin I Nitrogen	Pseudoglobulin II Nitrogen	Total Globulin	Albumin Globulin
Adult						
1	1.379	0.254	0.363	0.070	0.687	0.540
2	1.322	0.223	0.232	0.193	0.648	0.492
3	1.340	0.236	0.193	0.122	0.551	0.532
4	1.167	0.247	0.102	0.100	0.449	0.421
5	1.423	0.280	0.158	0.109	0.547	0.408
New-born infant, umbilical cord blood						
1	0.689	0.006	0.122	0.079	0.207	0.445
2	0.972	0.000	0.390	0.013	0.403	0.528
3	0.788	0.009	0.170	0.090	0.269	0.443
4	1.060	0.000	0.324	0.105	0.429	0.587
5	0.854	0.000	—	—	0.383	—
6	1.064	0.005	—	—	0.405	—
Infants, with colostrum						
3 weeks	0.983	0.206	0.222	0.087	0.515	
13 days	0.887	0.090	—	—	0.1407	
11 days	0.887	0.089	—	—	—	
Infant, no colostrum						
3 weeks	0.885	0.082	0.206	0.098	0.386	

that such passive immunity as very young animals exhibit does not come ordinarily, if at all, by transfer through the placenta, but by alimentary absorption from the colostrum.

The various facts cited above all lead to the conclusion reached by Famulener on the basis of a study of the attainment of the immune bodies by the new-born mammal, and also later by Wells and Osborne from a consideration of the chemistry of the milk proteins, that the formation of colostrum is for the purpose of presenting to the new-born mammal a concentrated solution of serum globulin which carries antibodies from the maternal blood, during the brief time that these protective proteins can be absorbed unaltered from the alimentary canal in an active condition.

An important chemical contribution to this subject, which harmonizes perfectly with the foregoing hypothesis, has been made by Howe,[9] who found that the blood of a new-born calf does not contain euglobulin, this being the globulin fraction of serum with which the antibodies are found associated. If the calf suckles a cow secreting colostrum, the blood serum soon contains relatively large amounts of euglobulin; but if the calf suckles a cow that has been lactating for some time, and yielding milk which is known to contain very little globulin, the quantity of euglobulin in the calf's blood remains negligible for several hours, and it increases much more slowly than in a calf that has received colostrum from the beginning.

The practical importance of the foregoing observations on the lower mammals has led us to make observations on the human subject, and we have repeated Howe's investigations on the blood of adult persons and on young infants, using the method which he devised for the quantitative estimation of the protein fractions in small quantities of blood. The results are given in the accompanying table, the figures being grams of nitrogen per hundred cubic centimeters of serum.[10]

Examination of this table discloses that the newborn human infant is virtually devoid of euglobulin in its blood serum, although it has quite as much serum albumin and about the same quantity of pseudoglobulin as the adult. This is in striking contrast with the practically constant presence of about 0.225 to 0.250 gm. of euglobulin nitrogen per hundred cubic centimeters in the blood serum of adults. Furthermore, we see in the serum of young infants a gradual rise in the euglobulin content, more rapid in infants receiving colostrum as far as can be ascertained from the few figures available, the number being limited by the difficulty of securing suitable material. Therefore, we have found in human infants exactly what Howe found in calves, namely,

9. Howe, P. E.: An Effect of the Ingestion of Colostrum upon the Composition of the Blood of New-Born Calves, J. Biol. Chem. **49**: 115 (Nov.) 1921.

10. The blood of the new-born infant was obtained from the umbilical vein. When free hemoglobin was present in the serum it was found that the results for euglobulin were too high, and such specimens were discarded. The blood of the infants was obtained from the superior longitudinal sinus. We are indebted to Dr. E. L. Cornell of the Chicago Lying-in Hospital, and Dr. W. B. Moody of the Cook County Hospital for our material.

that they are born with no euglobulin in their blood, and that they acquire it, at least in large part, from the colostrum.

Why the other blood proteins are present in the serum of the new-born animal in about the same proportions as in the adult, while the euglobulin alone has to be obtained after birth, we cannot explain. The fact indicates at least that the euglobulin is in some way essentially different from the other blood proteins. Another respect in which euglobulin is unique is its association with the immune bodies of the blood. These two peculiarities, added to the fact that euglobulin is the predominant protein of colostrum, being the only blood protein secreted by the mammary gland into the milk, seem to establish a new significance to this protein, or this fraction of the serum proteins.

These findings all add force to the conclusions expressed by Famulener in these words:

> All observers are agreed in the conclusion that the newly born animal (or infant) absorbs from the digestive tract unaltered antibodies most readily when given immediately after birth. The most effective means of supplying the antibodies to the young is through the mother's colostrum-milk (homologous); probably next in importance is the homologous serum, and finally the antibody milk or serum from some other species. It follows from our observations that of all factors the colostrum plays the most important rôle in transmitting antibodies from the actively immunized mother to the young. Even under normal conditions, it is reasonable to suppose that protective substances are concentrated in the colostrum from the mother's body fluids and may be in part absorbed by the infant after the first feedings. Consequently *it seems of highest importance that the newly born child should, during the first days of life, receive the colostrum-milk* [italics ours] unless it is contraindicated for some special reason. Perhaps the natural resistance which many infants show against many infectious diseases may be partially explained on the basis that the child is passively immunized by antisubstances excreted through the immune mother's colostrum and milk, which are absorbed by nurslings during the early days of life. Such a view gains strength from the observations of Moro, who found that the blood serum from breast-fed infants showed greater bactericidal and hemolytic powers than that from infants who were artificially fed upon cow's milk.

The possibility of adding to the immune-body supply of the new-born infant by feeding serum or globulin concentrates rich in useful antibodies, or colostrum

from cows or goats, suggests itself, and is now the subject of our investigation. Even if this is possible it is very probable that it is not desirable, since, if the globulin which bears the immune antibodies is absorbed by the infant, there will presumably also be a sensitization to these foreign proteins which would certainly not be desirable, especially since any immunity thus acquired would be passive and therefore transient, while the sensitization would be active and permanent. The experiments of Wells [11] have shown that young animals, even after the first days of life, may be made hypersensitive to proteins given in their food, so that they give anaphylactic reactions when these proteins are injected into them. The temporary increase of passive immunity against infections to which the infant might never be exposed would probably not be worth the price of a possible horse asthma or food asthma, or a hypersensitivity to serum needed later for therapeutic purposes.

SUMMARY

The blood of new-born infants, and probably of all other mammals, contains little or none of the serum protein or protein fraction known as the euglobulin. This seems to be supplied chiefly by the colostrum, which differs from milk in containing a large amount of this protein secreted directly from the blood. The euglobulin is the only blood protein that appears in the colostrum, and it is the only protein fraction in which the new-born infants' blood is deficient. Evidently the colostrum is formed to provide the fetus with a supply of euglobulin for its blood, during the short period immediately after birth when proteins may be best absorbed without disintegration by digestive proteolysis. The importance of this lies in the fact that the protective antibodies of the blood are found associated with the euglobulin fraction, and that the quantity of protective antibodies found in the colostrum, the milk and the infants' blood varies directly with the euglobulin content of these fluids. Evidently the colostrum furnishes to the new-born mammal protective antibodies, which probably add much to its capacity to resist infection in early life. If the infant does not receive

11. Wells, H. G.: Studies on the Chemistry of Anaphylaxis, III, Experiment with Isolated Proteins, Especially Those of the Hen's Egg, J. Infect. Dis. **9**: 147, 1911.

colostrum it acquires euglobulin in its blood much more slowly, and presumably is in corresponding degree less resistant to infection. It is not probable that there is any equivalent substitute for human colostrum for new-born infants. The findings of numerous recent workers reviewed in this article add force to the statements made by Famulener, ten years ago, that it is most highly desirable that every new-born infant should receive its full ration of human colostrum

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The Racial Distribution of Isohemagglutinin Groups

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THE RACIAL DISTRIBUTION OF ISOHEMAGGLUTININ GROUPS *

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According to our present understanding of human isohemagglutinins, there exist in the blood two types of specific agglutinating substances, which may be designated a and b. In the red cells there are two specific types of agglutininogenic substances, which may be called A and B. The agglutinin of Type a will agglutinate only cells containing the agglutininogenic substance A, and Agglutinin b will agglutinate only cells containing Agglutininogen B. Obviously, one type of agglutinin will occur only in the absence of its corresponding agglutininogen, a fact utilized in classifying samples of blood. A given specimen of blood may contain either one of these specific agglutinins, or both, or neither. This distribution of agglutinins and agglutininogens makes it possible to divide all samples of human blood into four different groups.

One group contains both types of specific agglutininogens, but the serum contains no agglutinins. A second group contains Agglutinin b and Agglutininogen A. A third group contains Agglutinin a and Agglutininogen B. A fourth group contains neither agglutinin but contains both agglutininogens. There have been various designations of these four groups but, because of the resulting confusion and the accidents that occurred in the practical application of blood grouping to transfusion, the American Association of Pathologists and Bacteriologists and the American Association of Immunologists have adopted officially the classification as proposed by Jansky,[1] because of its logic and priority. The groupings that have been

* From the Otho S. A. Sprague Memorial Institute, and the Department of Pathology, University of Chicago.

1. Jansky: Sborn. klin. **8**: 85, 1907.

proposed by von Dungern and Hirschfeld[2] and by Moss[3] differ from that of Jansky only in terminology. The first of the foregoing groups is called Group I; the second, Group II; the third, Group III, and the fourth Group IV. This is also the numerical order in which they occur in human bloods. The Jansky grouping is presented schematically in Table 1.[4]

The study of the origin, distribution and significance of these as yet little understood substances will undoubtedly prove interesting. According to H. and L. Hirschfeld,[5] who examined about 8,000 samples of blood from the various races assembled in Europe during the recent war, the distribution of the four groups of hemagglutinins among these people varied extremely,

TABLE 1.—JANSKY BLOOD GROUPING

Group	Effect of Serum on Other Groups	Effect of Other Groups on Cells
I	Agglutinates cells of the three other groups; contains Agglutinins a and b	Inagglutinatable; contains no agglutininogen
II	Agglutinates cells of Groups III and IV; contains Agglutinin b	Agglutinated by serum of Groups I and III; contains Agglutininogen A
III	Agglutinates cells of Groups II and IV; contains Agglutinin a	Agglutinated by serum of Groups I and II; contains Agglutininogen B
IV	No agglutinative effect; contains no agglutinins	Agglutinated by serum of Groups I, II and III; contains Agglutininogens A and B

but certain races could be grouped together because of the similarity in the distribution among them of the specific agglutinins a and b. This grouping of races corresponded somewhat to the grouping made on an anthropologic basis; it corresponded still more completely to the geographic distribution of the races. It was found that the European type, including Englishmen, Frenchmen, Italians, Germans, Austrians, Bulgarians, Serbians and Greeks, is characterized by the prevalence of Group II of the hemaglutinins and a low incidence of Group III. Among the Asio-African group, including Malagasies, Negroes, Anameses and Hindus, the incidence of Group II is low, while that

2. Von Dungern and Hirschfeld: Ztschr. f. Immunitätsforsch. u. exper. Therap. **8**: 526, 1911.
3. Moss, W. L.: Bull. Johns Hopkins Hosp. **21**: 67, 1910.
4. Ottenberg, Reuben J. Immunol. **6**: 363 (Sept.) 1921.
5. Hirschfeld, H., and Hirschfeld, L.: Lancet **2**: 675 (Oct. 18) 1919.

of Group III is high. There is an intermediate type including Turks, Arabs, Russians and Jews, in which the two groups occur intermediate to that of the European and Asio-African types. The proportion of the prevalence of Agglutinin a and Agglutinin b in each race is expressed numerically as a biochemical race index. The race index for the European peoples varies between 4.5 and 2.5; for the Asio-African peoples it is 1 or less, while the intermediate type is characterized by a race index of from 1 to 2.

This is an important contribution to anthropologic methods because it is a characteristic with a biochemical basis and is therefore definite and susceptible of accurate study. Some of the uses that the method may serve are pointed out by the Hirschfelds. They seem to find evidence in their work that suggests two sources of origin of the human race, one in India, where Agglutinin a arose, and the other in western or central Europe, where Agglutinin b had its origin. It is believed that further examination of the northern races will lead to definite conclusions as to this view. The relations of races as indicated by the hemagglutinins are in some instances inconsistent with those previously established by the usual methods of anthropology. For instance, Russians and Jews, who differ so much from each other in anatomic characteristics, mode of life, occupation and temperament, and because of this have been thought to be in different anthropologic groups, have exactly the same proportions of Groups II and III, their race index being 1.3. The Hindus, who are considered anthropologically near the Europeans, show the greatest difference from them in blood properties. The race index of the Hindus is 0.5, compared with 4.5 of the English and 2.5 of the Greeks, the highest and the lowest of the European race indexes. It more nearly approximates the index of the Negro and Anamese, 0.8, to whom the Hindus have a resemblance in skin pigmentation.

Prichard [6] defines a race as a collection of individuals representing more or less common features transmissible by heredity. Isohemagglutinins, the distribution of which characterizes a race, seem to be a biologic character that is inherited in true mendelian fashion.

6. Prichard, in Cowles, James: Physical History of Mankind. Ed. 4. London, Sherwood, Gilbert & Piper **1**, 1842.

Von Dungern and Hirschfeld [7] first showed this to be true, and their findings have been amply confirmed by Ottenberg.[8] A given kind of specific agglutinin or agglutininogen never occurs in a child unless it is present in one of the parents, and when one of these substances is present in both parents it occurs in most of the children. When one of the substances occurs in only one parent, some of the children inherit it, and when neither parent has one of the substances no child ever shows it. This fact would seem to indicate that the findings of the Hirschfelds are not accidental but are true racial characteristics, the perpetuity of which is insured. Verzar and Weszeczky [9] found that several races (Germans, Slavs and Gypsies), living side by side in Hungary for several centuries but without intermingling, have retained their characteristic blood group distribution, it being identical with the distribution among the same races in other countries.

What happens on the intermixture of two races with different distributions of hemagglutinins has not been studied. Anthropologists, with their more established methods, are able to detect the derivation of races by isolating in them the characteristics which are known to belong to pure races. It might also be possible to determine the components of a given race from the study of the distribution of the hemagglutinin groups. As a prerequisite, one would desire to examine a mixed race, of which the constituents, as well as the distribution of hemagglutinins among these constituents, are known. Such an opportunity presents itself in America, where there has been a widespread intermixture of Negroes and Caucasians, both of whose biochemical indexes are known. The amount of such intermixture is difficult to estimate, but it is unusual to find an individual of African descent who does not show, or give a history of, intermixture with the Caucasian race.

As pointed out by Reuter,[10] the inheritance of Negro characteristics in Negro-white crosses overwhelms, and to a great extent obscures, many of the inherited white

7. Von Dungern and Hirschfeld: Ztschr. f. Immunitätsforsch. u. exper. Therap. **6**: 284, 1910.

8. Ottenberg, Reuben: Medicolegal Application of Human Blood Grouping, J. A. M. A. **77**: 682 (Aug. 27) 1921.

9. Verzar, F., and Weszeczky, O.: Biochem. Ztschr. **126**: 33, 1921-1922.

10. Reuter, E. R. The Mulatto in the United States, Boston, R. G. Badger, 1918.

characteristics. Some years ago Dubois[11] estimated that one third of the American Negroes had some white blood. Because of the obscured intermixture and because of the constant increase, it is very probable that the present figure is greater than that given by Dubois. It would seem, then, of interest and of some possible importance to study the effect, on the distribution of hemagglutinins, of the intermixture of these two races which are anthropologically different and among which the hemagglutinin groups occur with such wide difference.

Blood samples from 270 Negroes were examined, and the group number for each determined. At first an attempt was made in each case to estimate the amount of white intermixture from the characters which are supposed to vary with the degree of intermixture, such as pigmentation, texture of hair and features. It was soon found that this was impossible, as these characters did not vary equally and quantitatively.

As among other groups of people, Group I of the hemagglutinins predominated. Forty-nine per cent. were found to belong to this group, which is larger than that found for other races. Group IV was the smallest, 5.53 per cent.; 26.9 per cent. belonged to Group II, and 18.4 per cent. to Group III. Table 2 presents the results in tabular form, for comparison with the results of other investigators.

Table 2 shows that the percentage of Group II in American Negroes is much lower than in white persons, while Group III is much larger in the Negro. This finding, though approximating the findings of the Hirschfelds for African Negroes, differs from them in that the Group II figure is higher and the Group III figure is lower. In other words, the percentages of these two groups tend to approach those for white persons.

The Hirschfelds use, as the race index, the relation of the percentage of Agglutinin b (Groups II and IV) to the percentage of Agglutinin a (Groups III and IV). The index for African Negroes is 0.8. The index for American Negroes, as calculated from our results, is 1.4.

11. Dubois : Atlanta University Publications, No. 11.

As no other factor is known which may change the distribution of hemagglutinin groups among a race, it is probable that the change that has been observed for negroes is the result of intermixture with the white race. It is all the more probable because the change in figures has been in the direction toward the figures for the white persons.

TABLE 2.—BLOOD GROUPS IN THE NEGRO AND WHITE RACES

Investigator	Locality	Race	Number Examined	Groups I %	II %	III %	IV %
Hektoen *	Chicago	White	70	47	34	10	9
Moss [3]	Baltimore	White	100	43	40	7	10
H. and L. Hirschfeld [5]	England	White	500	46.4	43.4	7.2	3
H. and L. Hirschfeld	Africa	Negro	500	43.2	22.6	29.2	5
Lewis and Henderson	United States	Negro	270	49	26.9	18.4	5.5

* Hektoen, Ludvig: J. Infect. Dis. **4**: 297, 1907.

CONCLUSIONS

1. The distribution of isohemagglutinin Groups II and III among American Negroes is very different from that among the white race.

2. It approximates the distribution of hemagglutinin groups among African Negroes, but differs from the latter in an increase of the percentage of Group II and a decrease of the percentage of Group III.

3. Since the difference in the distribution of hemagglutinin groups among African and American Negroes lies in the direction of an approach to the distribution among the white race, it is assumed that the change is due to intermixture with the latter.

We wish to acknowledge our indebtedness to Dr. H. Reginald Smith, Provident Hospital, and Dr. Emmanuel B. Fink, Cook County Hospital, for furnishing the samples of blood used in these experiments.

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POST-TRAUMATIC CALCIFICATION OF THE PANCREAS, WITH DIABETES.

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The condition described in the above caption is, as far as some search through the literature seems to indicate, altogether unique, and hence a case illustrating it is herewith reported in some detail, especially since evidence bearing on the relation of the pancreas to diabetes is also of value.

The subject was a man, aged thirty-two years, a teamster, who while driving his wagon fell from the seat. As to whether the wheels of the wagon passed over his body or not the evidence of bystanders was conflicting, but from the nature of the injuries found at the autopsy there can be no doubt that they did. This happened on March 31, 1919, and he was at once taken to the Cook County Hospital. The examination on his admission showed him to be under the influence of alcohol, with the left sixth, seventh, eighth and ninth ribs fractured in the axillary line and considerable subcutaneous emphysema. No other injuries could be found, and, as far as the records show, no urinalysis was made. There was elicited a history of a fracture of an arm two years previously and of habitual alcoholism, but no evidence of any illnesses. He remained in the hospital until April 12, when he was discharged as recovered from the fractured ribs.

August 17, he was brought back to the hospital in an irrational, slightly stuporous condition, much emaciated and evidently seriously ill. It was learned that shortly after his return home a suppuration appeared on the inner side of the right elbow, which had not been observed to be injured while in the hospital, and from this there had been continuously a slight discharge of pus. His relatives stated that he had not been normal mentally since the accident, and he had been unable to work; he was irritable, stupid and made mistakes in conversation and in action. There was a

progressive and marked loss of weight, estimated at about twenty pounds, although he had been slender previously. For three weeks previous to his return to the hospital he had been having typical attacks of Jacksonian epilepsy, increasing progressively in frequency and severity. The onset of each attack was with a sensation of stiffening in the left foot, followed by twitching and jerking which spread up the left thigh to involve the abdominal and chest muscles, then extended to the right lower extremity, then both upper extremities, beyond which the patient has no recollection. Sometimes the attacks were at intervals of not over one-half hour.

Observed in an attack in the hospital the patient began by having clonic spasms of the left lower extremity, then he stiffened his trunk and his arms began to jerk, his hands half closed, the fingers extended and shook; his mouth was half-open, his lower jaw moving in jerks, his facial muscles twitched and his eyes rolled. At the beginning of one of these spasms the patient gave successive loud cries. The spasms continued for about one or two minutes, and then the patient relaxed in about fifteen to thirty seconds; he was almost immediately conscious after the jerking stopped. There was no previous history of scalp injury, except being hit with a beer bottle about five or six years before; the blow dazed him for ten to fifteen minutes.

The only other information obtained was that he had had a slight cough for about a year and that he had had gonorrhea but not syphilis. On entering the hospital he complained of weakness, loss of appetite, nausea and great thirst. His relatives stated that for some time after his accident he had been noted to be extremely thirsty, drinking large quantities of water. Also he had complained of itching.

Urine had been passed during the convulsions, and so no estimate of polyuria was obtainable.

The chief physical findings on readmission to the hospital were the following:

The patient is a white man of about thirty-two years of age, of medium height, emaciated and in a slightly stuporous condition. There is a thickening of the end of the humerus at the internal condyle, with a slight stiffness of the elbow. A sinus opens about 4 cm. above the lower portion of the internal condyle, discharging a small amount of seropurulent fluid.

Teeth. Carious.

Tonsils. Moderately enlarged; rest of head negative.

Neck. Negative.

Chest. Symmetric and emaciated; clavicles prominent. The semistuporous condition of the patient makes it difficult to obtain findings. Rales present over the whole chest, specially the lower lobes.

Heart. Apex not palpable. Left border ½ cm. from midline, right border substernal. Rate, 128. First sound weak, second pulmonic accentuated. No murmurs.

Abdomen. Retracted and tense. Liver dulness, sixth interspace to costal margin. Kidney and spleen not palpable. No masses or tender spots.

Genitalia. Negative.

Extremities. Negative except for right elbow as mentioned. Reflexes exaggerated on left side. Some spasticity on left side.

Clinical Diagnosis. Chronic osteomyelitis of the right lower third of the humerus. Jacksonian epilepsy of undetermined etiology.

During the forty-eight hours that the patient was in the hospital he had repeated convulsions, during which urine was passed, and as there were no special indications of urinary change, no effort was made to secure specimens, so we have no record of the composition of the urine. The Jacksonian epilepsy dominated the picture completely, and he died in *status epilepticus* on August 20, and an autopsy was performed eight hours after death.

The autopsy findings were as follows:

External Appearance. The body is that of a small, poorly built, poorly nourished white man. *Rigor mortis* in legs and fingers. No edema. No icterus. No adhesions between the scalp and the skull. No evidence of trauma to head. Superficial lymph glands not enlarged. External genitals normal. No subcutaneous fat. On the inner aspect of the right elbow there is an elliptical opening in the skin, 10 by 20 mm., with a granulating floor, and the skin is much undermined. When cut through there is a superficial suppurating area between the skin and the muscle fascia anterior to the internal condyle, extending down into the internal condyle, where there is a suppurating cavity 1 cm. in diameter.

Abdominal Cavity. The inguinal and femoral rings are closed. Omentum is free from fat and adherent about the cecum. There are small hard lymph glands in these adhesions. The appendix lies behind the cecum and is remarkedly kinked on itself, forming a T, and is bound down by adhesions. There are also numerous vascular tags of adhesions over the sigmoid and elsewhere on the intestines. The mesenteric lymph glands are all slightly enlarged, pale and hard. The surface of the bladder is covered by a vascular connective tissue. There are numerous folds and bands of adhesions throughout the pelvis. The omentum is black from pseudomelanosis. There are dense fibrous adhesions about the spleen. There are also a few adhesions about the gall-bladder, elsewhere the liver is free. The peripancreatic glands are slightly enlarged. The retroperitoneal glands are normal in size.

Pleural Cavity. The left pleural cavity is partly obliterated, laterally and over the base, by dense fibrous adhesions. The right

pleural cavity is completely obliterated. When the sternum was removed small pockets of pus were seen between the diaphragm and the lower margin of the right lung and chest wall. There is a hard white lymph gland present in the anterior mediastinum 1 cm. in diameter. Healed fractures of the sixth and seventh ribs can be felt on the left side at the anterior axillary line, with some overriding of the ends. Here the pleural adhesions are most dense. The lymph glands about the hilum of the left lung show small suppurating areas. There are numerous (twenty to thirty) small pockets of thick greenish-yellow pus lying beneath the parietal pleura on the right side, irregularly placed all over the cavity, in size up to 2 cm. in diameter. There are also a few such cavities within the pleural adhesions, specially between the diaphragm and the chest wall. No abscesses are found outside of the chest cavity or on the left side. There are no old fractures or other evidence of trauma on the right side. No subdiaphragmatic lesions.

Pericardial Cavity. Contains normal amount of fluid. There are protruding hyperemic areas showing through the left lateral aspect of the pericardium over the subpleural abscesses. The lining is smooth and not involved by this process.

Heart. Slightly enlarged. The left ventricle takes up the greatest area. There are small beaded areas in the coronary arteries. The right and left posterior cusps of the aortic valves are fused to each other by a soft fibrinous adhesion. The beginning of the aorta is nearly free from sclerosis. There is very little sclerosis in the thoracic aorta. Ductus arteriosus is closed. The tricuspid and mitral orifices are of normal size. The tricuspid and pulmonic valve cusps are normal. Along the free margin of the mitral valve, throughout its circumference, is a fresh growth of recent soft vegetations 1 to 3 mm. thick. Foramen ovale is closed. The walls of the ventricles are of normal thickness. The left ventricle is stopped in diastole. The myocardium is slightly mottled in appearance; there are no definite macroscopic changes. The empty heart weighs 300 gm.

Trachea. Normal.

Peribronchial Lymph Glands. No caseation or calcification; some contain small suppurating foci.

Esophagus. Normal.

Lungs. The left lung is inelastic and there are no definite areas of consolidation; weight, 600 gm. The lower lobe is very dark in color and contains much fluid. Every bronchus when cut is filled with creamy pus. The upper lobe shows the same condition, particularly in the posterior portion, less marked anteriorly. The right lung is covered with adhesions. Weight, 840 gm. There are some hard scars in the apex, especially the posterior part. Abscesses as described above are noticed in the pleural adhesions.

There are also some small areas of hemorrhage in the pleura. Section through the upper lobe shows a distinct cavity 18 to 20 mm., running from which are lines of scar tissue. There is a caseous nodule at the upper apex 10 by 12 mm. Another small cavity is found in this upper lobe. There are some small gray tubercles in the peribronchial lymph gland that receives the drainage from the right upper lobe. There are no other definite areas of consolidation.

Spleen. Embedded in adhesions. Weight, 70 gm. The upper pole alone remains. The lower pole is hard, yellow, shrunken and covered with adhesions. When cut the entire lower pole is found to be replaced by a well-defined encapsulated necrotic mass, 5 x 4 x 3 cm. The rest of the spleen is very soft and pulpy.

Kidneys. The left kidney is surrounded by a mass of flabby fibrofatty tissue which is very adherent to the kidney. There is a total infarction of the left kidney. The size of the left kidney is approximately $5\frac{1}{2}$ x 4 x $1\frac{1}{2}$ cm. The structures are left in recognizable form but yellow and completely necrotic, without suppuration. The left renal artery is much smaller than the right and completely obliterated at the hilum of the kidney. Right kidney large, weight, 200 gm., soft. Cut surface shows cortical markings distinct. Cortex thick, 8 to 10 mm. There are no signs of abscesses. The capsule strips readily leaving a smooth surface.

Adrenals. Right adrenal seems normal in size and appearance. Left adrenal is normal except for one island of white tissue (5 x 10 mm.) in the medulla.

Liver. Dark in color and soft. Weight, 1900 gm. The cut surface shows considerable blood. The center of the lobules are dark. The cut surface is very wet. No other changes. Gallbladder: Normal. No concretions.

Pancreas. More rounded and thin than normal. Weight, 122 gm. The irregularity increases from head to tail. Head is distended with a mass the size of a hen's egg. Upon palpation the entire pancreas is full of small hard lumps, feeling as if filled with small stones. These concretions can be felt throughout the pancreas, about equally in all parts. They are all embedded in the tissue and a watery purulent fluid exudes from the ducts. They are not loose in the duct but are in the tissue itself, mostly firmly adherent; a few protrude into the duct, apparently by erosion from the outside. There are areas of partial stenosis along the pancreatic duct, but it is not anywhere completely obstructed. A few small concretions are found free in the duct. The entire pancreas is composed of scar tissue and calcified masses, no normal gland tissue being found. There is an increase in the peripancreatic fibrous tissues, but no concretions are found outside the gland tissue.

Stomach. Postmortem digestion. No lesion. Intestines: Normal.

Urinary Bladder. Greatly thickened with some hypertrophy of trabeculæ. Mucosa normal. *A small quantity of wine was present which contained* 2½ per cent *of sugar.*

Prostate. Somewhat enlarged and forms a ring about the urethra. There are no other gross changes. Seminal vesicles normal.

Testicles. Both normal.

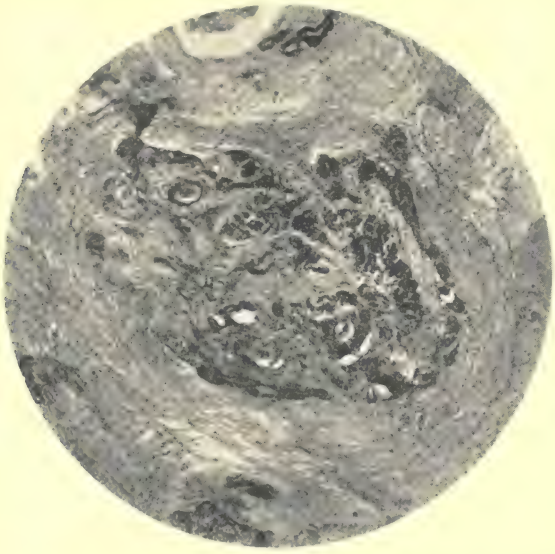
Skull. The calvarium is normal. The dura on the external surface appears normal. There is no undue tenseness. There is a considerable amount of cerebrospinal fluid both between the dura and pia arachnoid and in the pia arachnoid. This fluid is clear.

Brain. Weight, 1500 gm. There is a diffuse reddish discoloration of the pia over the right lateral aspect, particularly at the junction of the frontal and parietal lobes. Elsewhere the pia is clear. There is an area of softening 4½ cm. long by 2 x 2½ cm. wide, involving the ascending frontal and ascending parietal convolutions in this area, the upper margin of the softening being 3 cm. from the midline. There is no evidence of suppuration in the meninges. The softened area averages 5 to 10 mm. thick, involving the gray matter chiefly. The lateral ventricles are of normal size, containing a clear fluid. The basal ganglia appear normal.

Anatomical Diagnosis. Infarction with softening of the right ascending frontal and parietal convolutions. Multiple subpleural tuberculous abscesses in the right chest wall. Bilateral hypostatic congestion and acute suppurative bronchitis. Bilateral adhesive fibrous pleuritis. Acute terminal mitral and aortic endocarditis. Complete anemic necrosis of the left kidney with compensatory hypertrophy of the right kidney. Healed anemic necrosis of lower three-quarters of the spleen. Extensive fibrosis and calcification of the entire pancreas. Pancreatic diabetes (glycosuria). Healed fractures of the sixth and seventh left ribs. Abscess in internal condyle of right humerus with fistulous cutaneous opening. Parenchymatous degeneration of the liver and the right kidney. Hypertrophy of the urinary bladder wall. Healed appendicitis. Ulcerative and fibroplastic tuberculosis of the right pulmonary apex. Suppurative tuberculous mediastinal lymphadenitis. Hyperplastic tuberculosis of the mesenteric lymph glands. Healed necrotic area in the left adrenal. Healed diffuse peritonitis.

Histological Examination. *Pancreas.* The pancreas shows an extreme grade of fibrotic pancreatitis. (See Figure.) A small number of isolated, shrunken pancreatic islets remain, many being partially fibrosed. Besides these there are only traces of pancreatic tissue, although occasionally necrotic remains of pancreatic gland cells are found, barely recognizable. There are innumerable areas

of calcification throughout the organ, each surrounded by dense fibrous tissue. The amount of replacement by fibrous tissue is so great that it is impossible to determine whether the calcific deposits are exclusively intra-alveolar or not, but certainly most of the calcium is deposited in the sites of necrotic glandular masses. Besides the large calcified masses the tissue is sprinkled everywhere with minute quantities of calcium salts, replacing small groups of dead epithelial cells. No calcification of the vessels or of the fibrous septa is observed. Occasional islands of fatty areolar tissue remain within the fibrous masses, but these are never found calcified. No blood pigment is found.



Traumatic pancreatitis. This was one of the few islands of pancreatic tissue still present, located near the duct in the head of the pancreas. As will be observed, it consists chiefly of duct-like tubules rather than secretory structures, and these are isolated and compressed by scar tissue. Magnified 45 diam.

Among the few small foci of recognizable glandular tissue that remain are many that consist of cells that have lost their nuclear staining and are surrounded by very heavy masses of fibrous tissue. A little pancreatic tissue that seemed capable of functioning is seen in only a few of the several parts of the gland that were examined, chiefly about the ducts near the head of the pancreas. These consist of the remains of lobules, with a dense fibrous capsule about them, fibrous tissue infiltrating between the atrophic tubules and ducts that remain, and occasionally a small atrophic Langerhans

islet is recognizable. Nowhere is any area of even approximately normal pancreatic tissue to be found. It would seem that over 95 per cent, and probably fully 99 per cent, of the secreting pancreatic tissue has been replaced by fibrous tissue and calcium salts. There are no evidences of inflammation present, either tuberculous or acute.

Heart. The valves show recent vegetations. The myocardium is normal.

Lungs. The lungs show both old encapsulated and active tuberculosis, as well as acute hypostatic pneumonia and acute bronchitis.

Lymph Glands. The glands, particularly the intrathoracic glands, exhibit caseous and encapsulated tuberculosis, often with softening. The mesenteric and retroperitoneal glands show small tubercles, with much endothelial hyperplasia.

Adrenals. The left adrenal gland exhibits an extensive fibrotic area at the center, containing considerable blood pigment, apparently representing the site of a healed traumatic lesion. Elsewhere the adrenals are normal.

Liver. Shows no changes.

Kidneys. The right kidney shows a distinct increase in the size of the glomerules and tubules, without other noteworthy changes. The left kidney is completely necrotic, without sign of infection. Much thickening of the pericapsular tissues. The original structures are all visible but totally unstained.

Brain. Shows an old infarct with abundant blood pigment in phagocytes. The absorption of the necrosed tissue seems to be complete.

Spleen. The necrotic portion resembles a typical old infarct, with abundance of blood pigment, both granular and crystalline. The remaining living portion shows large Malpighian bodies and some blood pigment in the pulp.

Chemical Analysis of the Pancreatic Tissue. Samples from several parts of the alcohol-fixed gland were selected, to give a fair average of the composition, and the inorganic material determined. In 0.9546 gm. of dried material was 0.6100 mg. of inorganic residue, or 63.9 per cent.

Several of the calcified masses were picked out of the pancreas and separated as completely as possible from tissue elements. They were analyzed for me by Miss Mary E. Maver, using the wet method used in my previous work on calcification, which method avoids the errors introduced by ashing.[1] Two such lots were analyzed at different times, and the following results were obtained:

[1] Wells: Jour. Med. Res., 1906, **14**, 491.

PANCREATIC CALCIFICATION.

	Sample I.		Sample II.		
	Gm.	Percentage of inorganic.	Gm.	Percentage of inorganic.	Average per cent.
Ca	0.3765	35.6	0.1110	38.9	37.25
Mg	0.0028	0.27	0.0028	0.9	0.63
CO_2	0.2725	25.9	0.0696	24.2	25.05
P	0.0141	1.33	0.0008	0.27	0.80
Theoretically the inorganic elements existed as follows:					
$CaCO_3$	0.8848	92.7	0.2738	95.20	93.95
$Ca_3(PO_4)_2$	0.0585	6.1	0.0040	1.39	3.74
$MgCO_3$	.....	.....	0.0098	3.4	
$Mg_3(PO_4)_2$	0.0103	1.08			

From these figures it is seen that the concretions consisted chiefly of calcium carbonate with but small quantities of calcium phosphate and magnesium salts. About 20 per cent of the calcium found could not be balanced with acid radicals. Search was made for oxalic acid and fatty acids, with which the calcium might have been united, but neither was obtained. It is observed that the analysis of two different lots agreed quite closely as far as the CO_2 was concerned, so it was certain that there was no loss in this determination. Thorough extraction with boiling amyl alcohol failed to dissolve out any calcium soaps. No silicate was present, a point of some interest, since some German writers have laid stress on the function of the pancreas as a storehouse for silicates and as a center of silicic acid metabolism.

This high proportion of calcium carbonate is entirely different from the figures usually obtained in analysis of calcified tissues, in which generally about 85 per cent of the inorganic material is calcium phosphate and about 10 per cent is calcium carbonate. On the other hand our figures agree closely with some of the figures given for calculi found in the pancreatic *ducts*. Thus, Möckel,[5] in his compilation on pancreatic calculi, gives the following figures reported in the literature: I, $CaCO_3$, 91.65 per cent (Johnston, Moynihan). II, $CaCO_3$, 82.0; $Ca_3(PO_4)_2$, 12.7 per cent (Maurice, Lussac). III, $CaCO_3$, 93.14 per cent; P_2O_5, 2.45 per cent (Legrand, Scheunert). Other analyses of pancreatic calculi, however, have shown the chief constituent to be calcium phosphate. Presumably the predominance of calcium carbonate in pancreatic calculi, as well as in this one specimen of calcification of the pancreatic tissue, depends on the fact that the pancreas secretes a fluid alkaline with carbonates, since we find that the salivary duct

calculi also sometimes consist chiefly of calcium carbonate, although these also may at times contain chiefly calcium phosphate.[2]

Discussion. The history and the autopsy findings establish this as a case of post-traumatic calcification of the pancreas with diabetes. The fractured left ribs, the traumatic infarction of the left adrenal, the left kidney and the upper pole of the spleen indicate that the changes of the pancreas were also the result of traumatism to the left side and the upper abdomen. Probably the wheel of the wagon went across the lower chest and the upper abdomen, since merely a fall from this height could scarcely have produced this amount of injury in these deep-seated organs. The history of great thirst, pruritus and progressive emaciation after the accident, together with the presence of sugar in the urine and the very extensive destruction of the pancreas, establish the diagnosis of traumatic pancreatic diabetes. The acute tuberculous changes in the pleura and lymph glands may be the result of the diabetes, although it is impossible to determine that they may not have been incited by the trauma in a subject with an existing pulmonary tuberculosis. Also, it is not certain whether the area of cerebral softening that caused the Jacksonian epilepsy was the result of the fall or had come from embolism; the histology of the brain lesion, however, suggests that it was contemporaneous with the lesions in the spleen, kidney and adrenal, and the history stated that the patient had never been normal mentally after the accident. The endocarditis was distinctly a terminal infection and too recent to have caused the lesions in the kidney, spleen, adrenal and brain.

The calcification seems to be distinctly of the pancreatic glandular tissue itself and is not the result of pancreatic fat necrosis, for not only were there no calcified foci in the fat tissue outside the pancreas, but even the fat tissue within the pancreas itself showed no calcification.

It is unfortunate that the patient was not brought into the hospital until he was in a dying condition, and hence we have no data on the urine and the changes in the intestinal digestion that had resulted from so extensive a destruction of the pancreas.

Discussion of the Literature. As stated, no similar case can be found recorded. The extensive discussion of pancreatic cysts and concretions by P. Lazarus[3] makes no mention of calcification of the pancreatic tissue from any cause whatever nor of the occurrence of pancreatic calculi following trauma. He describes several cases in which scars and cysts have followed traumatism, but does not speak of calcification in connection with these. He also states that up to that time (1904) no case of diabetes had been described as a sequel of a traumatic cyst of the pancreas. Of interest in

[2] Roberg: Ann. Surg., 1904, **39**, 669.
[3] Zeit. klin. Med., 1904, **51** and **52**.

connection with our case is his statement that the most common cause of pancreatic trauma is being run over by vehicles and that the injuries are seldom of the pancreas alone, the liver, kidneys and spleen being often traumatized, as well as the ribs and the vertebral column. He also calls attention to the fact that only a part of the traumatisms of the pancreas are followed by fat necrosis, indicating that some added factor, such as infection or inflammation, must be present to produce this complication.

Opie in his monograph on *Diseases of the Pancreas* makes no mention of calcification of pancreatic tissues, nor does Kauffmann in his *Text-book of Pathologic Anatomy*. Numerous articles on pancreatic concretions have been reviewed, but in none of them do we find mention of calcification of the pancreas itself, except the statement in the article by Weichselbaum and Stangl[4] on the pancreas in diabetes that "in one case we demonstrated calcium deposits in the hyaline or sclerotic islands, whereby an increased similarity is given to sclerotic renal glomeruli." Neither is trauma mentioned as a cause of pancreatic calculus formation.[5]

While a complete search of all the literature on diabetes for cases of diabetes following trauma to the pancreas has not been possible because of the magnitude of the task a large number of articles and monographs have been reviewed, including practically all of the larger publications, virtually without finding any mention whatever of pancreatic trauma as a possible cause of diabetes. This is not strange in view of the fact that the pancreas is so well protected that only the most violent traumatism can injure it, and then the results are usually quickly fatal; if not fatal there is probably usually sufficient pancreatic tissue left to prevent diabetes. For example, Robeson and Cammidge in their work on the pancreas state that Garré was able to collect but 30 cases of pancreatic trauma, of which 3 recovered. Naunyn does not mention the possibility of diabetes as a result of trauma to the pancreas, but comments on the infrequency of diabetes following acute pancreatitis. Even the work devoted specifically to surgical diseases and injuries of the pancreas by Körte[6] makes no mention of calcification of the pancreas or post-traumatic diabetes. So, too, the works of von Noorden and Lépine are silent on this subject; the latter does mention traumatic diabetes as if depending entirely on nervous lesions, yet of 145 cases of traumatic diabetes collected by him in 8 the trauma is said to have been abdominal. Arnstein[7] in an elaborate review of 437 articles on chronic pancreatitis does not even mention either traumatic pancreatitis or calcification of the pancreas.

[4] Wien. klin. Wchnschr., 1902, **15**, 972.

[5] Literature given by Möckel, Frankfurter Zeit. f. Pathol., 1920, **24**, 78.

[6] Die Chirurgischen Krankheiten und die Verletzungen der Pankreas, Stuttgart, W. Körte, 1898.

[7] Centrl. f. d. Grenzgeb. d. Med. u. Chir., 1912, **15**, 90.

Jeanselme[8] reported, in 1897, 2 cases of bronzed diabetes, one of which, in a man aged forty-three years, was observed first a short time after receiving a blow near the umbilicus. The exact interval between the injury and the appearance of the symptoms of diabetes (marked thirst, anorexia, polyuria and loss of strength) is not given. It could not have been more than a few days, however, since it is said that the blow was received "at the beginning of the month of July," and the symptoms were said to have appeared about the 16th to 18th of the same month. Furthermore, nothing is said about the condition of the patient in the intervening time. Autopsy was performed on the fourth of the following January and disclosed the classic features of bronzed diabetes. The pancreas weighed 150 gm. and was irregularly pigmented. Microscopically it was sclerotic, although some lobules were little altered. Nothing was said in the autopsy report about the presence of any evidences of trauma in the abdominal cavity or elsewhere. With these scanty data it is not possible to prove that in this case the diabetes was the result of the abdominal traumatism, and it is highly doubtful that a traumatism to the pancreas could cause a bronzed diabetes in view of the evidence that in this disease the pancreatic sclerosis is the result of deposition of pigment in the pancreas.

In his work on the traumatic origin of internal diseases, Richard Stern[9] comments on the fact that in no published case of injury to the pancreas has glycosuria been observed.

We have found but one probably authentic case of post-traumatic pancreatic diabetes reported in the literature, by Grund.[10] The patient, a woman, aged twenty-five years, while six months pregnant, slipped on the ice and another person fell across her abdomen. After a period of abdominal pains and vomiting all symptoms subsided and a healthy child was delivered at term. A month later pain and vomiting recurred with icterus, there being intervals of good health, with the gradual development of mild diabetes. About a year after the trauma a pancreatic cyst was operated on. The icterus subsided at once and permanently, but the diabetes persisted and within a few months assumed a comparatively severe form. The metabolic findings are tabulated as recorded during the various phases of the case; they show that the functions of the pancreas in respect to the digestive processes were not essentially altered. At the time of the publication of this report the patient was still living, and so we lack anatomic proof of the pathologic changes present.

The only instance of calcification of the pancreas tissue itself of which we can find record, beyond the apparently trivial involve-

[8] Bull. et mém. de la Soc. méd. des Hôp. de Paris, 1897, 3d series, **14**, 179.
[9] Ueber traumatische Entstehung inner. Krankheiten, Jena, 1913, 2d ed.
[10] Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1907, **17**, 395.

ment mentioned by Weichselbaum and Stangl, has been reported by Bernhard Fischer,[11] who states that it is, as far as he knows, a condition never previously described. The subject was a youth, aged eighteen years, with a striking family history of diabetic relatives, who had typical juvenile diabetes terminating in coma. There was no history of any trauma mentioned. The autopsy disclosed nothing of significance outside the pancreas, which was of normal size (100 gm.) and color, somewhat indurated, and showing macroscopically on the cut surface numerous tiny white calcific points, compared to the calcified glomerules in senile kidneys. Microscopically the changes were remarkable, for not only were the number and size of the islands of Langerhans enormously increased, but most of them showed calcification, often extensive, as well as sclerosis and hyaline degeneration. Some of the islands were so enlarged as to resemble adenomas, one of them being 1.3 mm. in diameter, and the number was so great that there must certainly have been a new formation of islands. The calcium salts stained with silver nitrate, and hence were presumably phosphatic, at least in part. Fine droplets of fat were sometimes found in the calcified islands. The connective tissue was considerably increased throughout the pancreas, but the glandular parenchyma was little altered. There was some calcification in the internal elastic lamina and the inner layers of the media of the arteries, but none in the parenchyma or stroma of the pancreas outside the islands. There was nothing found to explain why this remarkable selective calcification of the islands had occurred. Presumably they had undergone hyaline degeneration and then this hyaline material had become calcified as hyaline deposits everywhere are prone to do, perhaps because of a physical affinity for calcium salts.

Of course in pancreatic fat necrosis the freed fatty acids unite with calcium to form soaps, which might perhaps become converted into phosphate and carbonate, although my own experiments indicate that the lesions of fat necrosis may disappear entirely within a short time,[12] and that implanted soaps are mostly reabsorbed rather than replaced by the less soluble phosphate and carbonate.[13]

Summary. A previously healthy young man fell from a wagon and was probably run over by at least one of the wheels. He received fractures of several ribs on the left side, traumatic infarction of the left adrenal, the upper pole of the spleen and the entire left kidney, together with a crushing injury of the pancreas, which resulted in almost complete destruction of the secreting elements, which were replaced by a large quantity of dense fibrous tissue and extensive deposits of calcium salts. Analysis showed that about 64 per cent of the dry weight of the pancreas consisted

[11] Frankfurter Ztschr. f. Path., 1915, **17**, 218.

[12] Jour. Med. Research, 1903, **9**, 70. [13] Ibid., 1907, **17**, 15.

of inorganic salts, chiefly calcium carbonate. Jacksonian epilepsy developed, the result of necrosis of a considerable area of the cerebral cortex, presumably occurring at the same time as the other injuries. The patient became rapidly emaciated, exhibited great thirst and the urine found in the bladder at autopsy contained sugar. A review of the literature has failed to reveal a similar case of extensive pancreatic calcification, whether post-traumatic or otherwise, or an analogous case of diabetes following crushing injury of the pancreas.

Primary Squamous-cell Carcinoma of the Kidney as a Sequel of Renal Calculi

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PRIMARY SQUAMOUS-CELL CARCINOMA OF THE KIDNEY AS A SEQUEL OF RENAL CALCULI*

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An unusual case of renal tumor that has recently come under my observation carries so much evidence as to the relation of trauma and tumor formation, as well as to other problems of pathology, that it seems worthy of publication. It concerns a case of primary squamous-cell carcinoma of the kidney, arising as a sequel of renal calculi.

REPORT OF CASE

History.—A man, aged 57, a tailor, entered the Cook County Hospital, June 13, 1921, two weeks before his death, complaining of abdominal pains of six months' duration, associated with frequent burning micturition, and a loss of about 30 pounds (13.6 kg.) in weight. There was no history of pain characteristic of renal colic, although he said that he had suffered from pain over the bladder when he was 20 years old; beyond this there had been no previous illnesses. Venereal infection and alcoholism were denied. He had never noticed blood in the urine, although it was turbid.

Examination.—The only positive findings on examination were these. On the right side of the abdomen there was a tender mass about the size of a coconut, extending from the costal border to 5 cm. above the umbilicus. Roentgen-ray examination revealed the presence of multiple stones in an enlarged right kidney. The cystoscope showed the vesical mucosa congested without ulceration. Ureteral catheterization showed that the right kidney did not function. With 1 c.c. of phenolsulphonephthalein, injected intravenously, the dye showed in the urine from the left ureter in six minutes, but none appeared from the right side in thirty minutes. The phenolsulphonephthalein test, intravenously, yielded 30 per cent. in the first half hour and 20 per cent. more in the second half hour from the left side. The urine contained albumin, pus, and blood, but few casts. A 25 per cent. solution of sodium bromid was injected by the ureteral catheters, the right side taking 20 c.c., the left 10 c.c. Roentgenograms then revealed, on the left side, a somewhat enlarged left kidney with slightly enlarged multiple calices; the right kidney showed a pelvis of irregular shape, apparently with escape of the injected fluid into the substance of the diseased kidney. The stones seemed to be not only in the kidney but also in the surrounding soft tissues.

Blood analysis gave the following figures: total nonprotein nitrogen, 39.27 mg. per hundred c.c.; urea nitrogen, 18.4 mg.; uric acid nitrogen, 2.27; creatinin nitrogen, 1.47. The leukocyte count was 11,800.

Following the ureteral catheterization the patient had a chill, and he continued to grow progressively weaker, usually with a subnormal temperature. On June 28, under local anesthesia, the right kidney was opened and three large

* From the Department of Pathology, University of Chicago, and the Otho S. A. Sprague Memorial Institute.

dendritic caculi, each about 3 cm. in diameter, were removed, together with large amounts of pus. The patient continued to sink, and died the next day, a postmortem examination being made about six hours after death.

Necropsy Findings.—The body was that of a medium sized man, with white skin showing no icterus. The superficial lymph glands were not enlarged. There was a recently sutured wound, about 14 cm. in length, in the back, below and parallel to the right twelfth rib. There were no other external abnormalities.

Abdominal Cavity: There was 1 cm. of subcutaneous fat in the midline. The mesenteric lymph glands were not enlarged. The lower pole of the left kidney extended slightly below the crest of the ilium. The peritoneal cavity was free from fluid and its surface was smooth and shiny. A tumor mass at the site of the right kidney extended to the anterior axillary line. The colon extended over and in front of the renal mass and was attached to the liver and mass by dense fibrous adhesions. A white nodule, 1 cm. in diameter, which resembled a neoplasm, was removed from the peritoneum in this region. When this was cut, it exuded a white, necrotic, softened material. There were no adhesions about the spleen or gallbladder.

Chest Cavity: There were no abnormalities. The mediastinal glands were not enlarged.

Heart, Aorta and Vessels: The heart was large in proportion to the weight of the body, weighing about 350 gm. The coronary vessels and their orifices were normal. There was no fibrosis of the myocardium. All valves were normal. The ascending aorta showed a few small yellow streaks which were more numerous in the arch than in the abdominal aorta. The lymph glands around the abdominal aorta were enlarged and continuous with the right kidney mass. The inferior vena cava was free from thrombi and compressed somewhat with lymph glands but it was not occluded. The left renal vein was compressed by lymph glands.

The trachea and esophagus were both normal.

Lungs: The lower portion of the right lower lobe was slightly boggy and contained little air; but there was no consolidation. There was an extensive calcification of one right peribronchial lymph gland. The weight of each lung was 400 gm. The lower lobe of the left lung contained small subpleural hemorrhages and a few small slightly consolidated areas over which the pleura was smooth and glossy. Yellow pus exuded from the bronchi in this region, together with a little fluid.

Spleen: There were no gross changes.

Liver: The liver weighed 2,120 gm. The organ as a whole was enlarged; the surfaces were rounded, moist, swollen and soft in consistency. There was no increase in connective tissue. The gallbladder was normal in size and contained no concretions.

Stomach: The stomach showed no gross abnormalities. Below the pyloric ring there were two pouches or diverticula, each about 1.5 cm. in size, in the duodenal mucosa, with no evidence of inflammation or ulceration.

Pancreas: This was normal.

Kidneys: The left kidney was larger than normal, weighing 300 gm. and measuring 14.5 cm. long, 7 cm. wide and 6 cm. thick . The organ was soft, mottled and irregular in outline with a nonadherent capsule which was attached to the perirenal tissue. The cut surfaces showed areas of marked disintegration, hemorrhage and softening with one abscess, 2 cm. in diameter, underneath the

capsule. The cortex was 10 mm. thick. The pelvis showed no ulceration. The ureters were of normal size and showed no ulceration but contained purulent material.

The right kidney weighed 800 gm. It measured 21 cm. long, 13 cm. wide and 7 cm. thick, and it was adherent to the perirenal tissue which was indurated and contained several abscesses up to 3 cm. in diameter. The kidney was firmly adherent to the perirenal tissue over the entire surface; and about the pelvis, extensions of tumor growth had produced nodules and strands of neoplasm in the perirenal tissue itself. The ureter was completely occluded at its junction with the pelvis, the lower portion of which was completely filled by masses of irregular cancer tissue, about 5 mm. thick, surrounding the entire

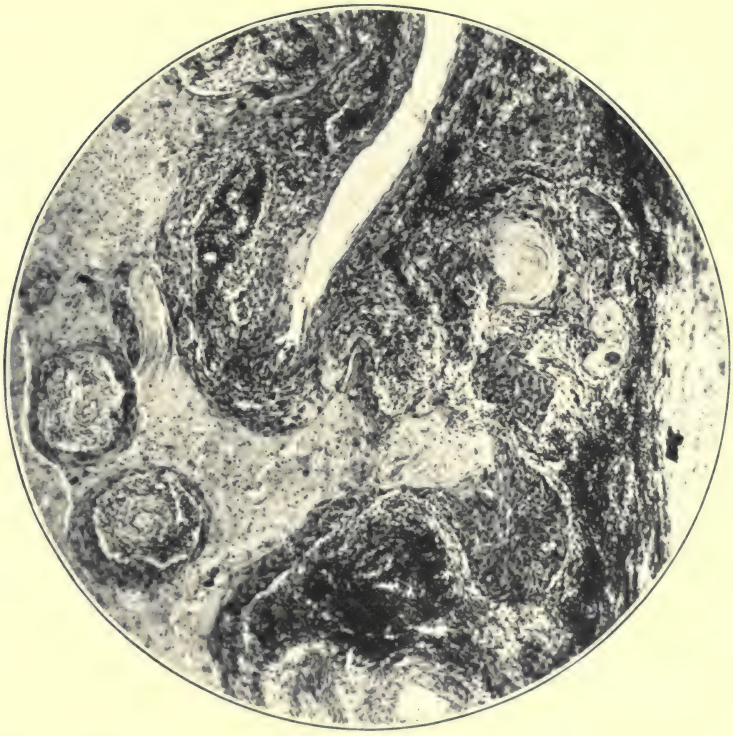

Fig. 1.—Squamous-cell carcinoma of the renal pelvis; × 60; showing the infiltration of the submucous tissues by carcinoma with formation of masses of keratin scales. The lining of the cavity is formed by stratified epithelium.

pelvis. The cut section of the kidney disclosed a complete loss of all recognizable kidney tissue. The organ consisted essentially of a mass of branching communicating cavities directly continuous with the pelvis, some of which extended to the capsule. The surfaces were bathed with thick pus in which a few small remains of the original calculus were found. Between the pus-filled cavities were masses of white cancer tissue with irregular necrotic surfaces, in places covered with blood clots. The cancer tissue infiltrated through the renal capsule into the perirenal fat in many places. The pelvic surfaces were

rough; but only occasionally was anything suggestive of a papillary character to be seen. In some places the shell of cancer tissue was only 1 or 2 mm. thick; in others, masses from 2 to 4 cm. thick remained. The kidney consisted practically of a pus-filled cavity formed by distention and ulceration of the renal pelvis, with walls composed entirely of cancer tissue. The portion of the stones obtained at the operation, together with the fragments found at necropsy, weighed 33 gm., air dried at room temperature. They constituted a typical dendritic calculus which had broken apparently spontaneously into three large fragments and four smaller ones. When fractured the surface was roughly crystalline. The external surface was lobulated, roughened, and showed some soft deposit on a harder central portion.

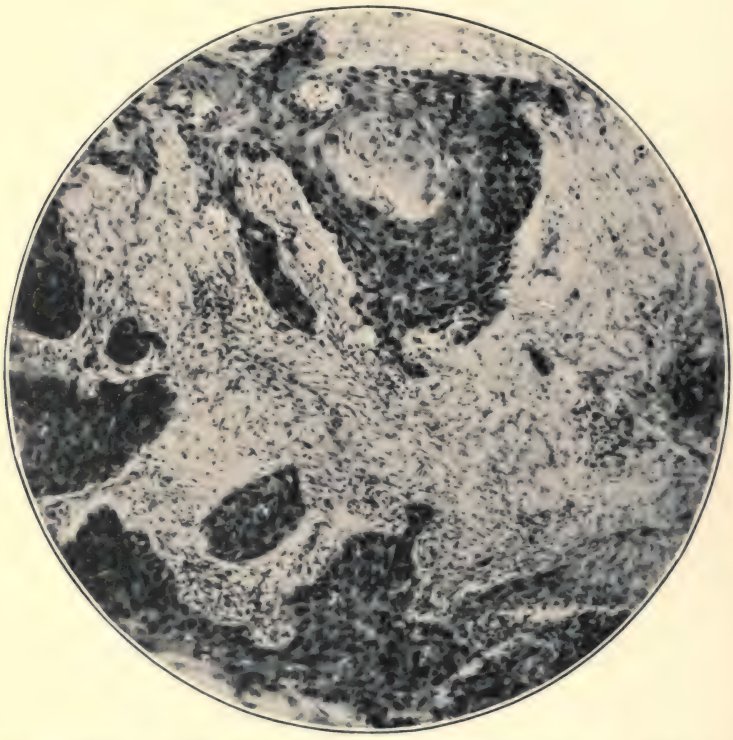

Fig. 2.—Squamous-cell carcinoma of the renal pelvis; × 110. Higher magnification to show the resemblance to squamous-cell carcinoma of the skin.

The lymph glands around the kidney were enlarged, up to 5 cm. in diameter, by white neoplastic tissue, with most of them showing suppurative softening.

Suprarenals: The left suprarenal was normal; the right was infiltrated with a white tissue, without much enlargement or nodule formation, apparently by direct extension from the kidney.

Bladder: The bladder contained a purulent material. The mucosa was hyperemic and contained much granulation tissue about the trigon, together with many small hemorrhages but no ulceration or tumor growths. Both ureteral orifices and the urethral orifice were normal.

The prostate, seminal vesicles and testicles were normal.

Skeleton: No metastases could be found. The vertebral column showed marked exostosis with complete ankylosis of many of the vertebral articulations.

Histologic Findings.—The white neoplastic tissue which replaced the right kidney and many retroperitoneal lymph glands, as well as the tumor nodule found in the peritoneum, consisted everywhere alike of typical squamous-cell carcinoma characterized by extensive formation of whorls of stratified keratinized epithelium. It differed not at all from the keratinizing carcinomas that are found in the skin or mouth. Nowhere were there structures resembling the ordinary papillary carcinomas of the renal pelvis, or the medullary transi-

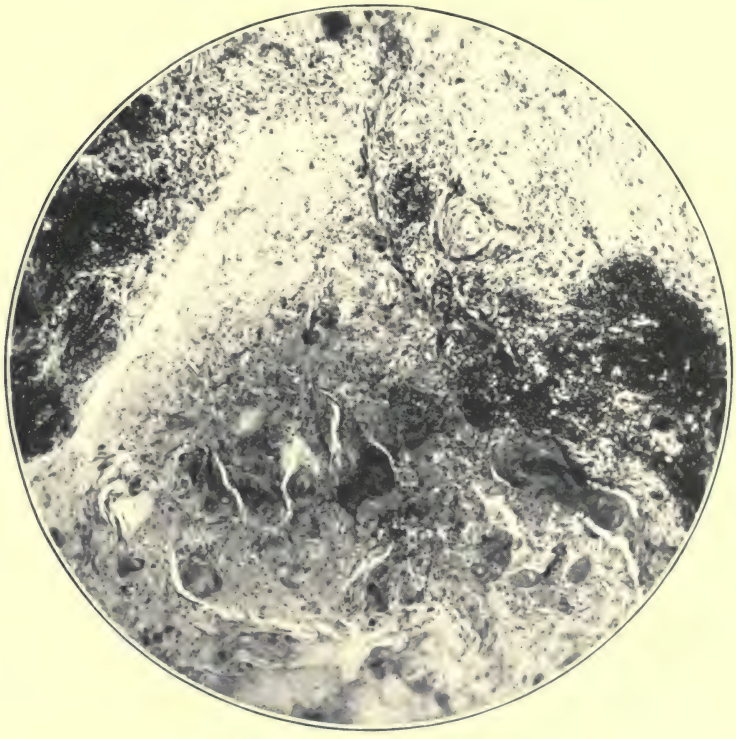

Fig. 3.—Metastatic squamous-cell carcinoma in a retroperitoneal lymph gland; × 60. Most of this field is occupied by masses of hornified epithelium.

tional cell carcinomas. In all parts of the tumor growth, both primary and secondary, there was acute suppurative inflammation. In none of several sections examined were any remains of the kidney tissue to be seen. The right suprarenal was partly replaced by carcinoma of the same type as that in the kidney.

The left kidney showed only the usual changes of an acute ascending suppurative nephritis.

No microscopic changes of present interest were found in the other organs.

Anatomic Diagnosis.—This was: right nephrolithiasis; diffuse infiltration of the right kidney and perirenal tissues with squamous-cell carcinoma; acute suppurative pyelonephritis with complete destruction of the parenchyma of the right kidney; recent nephrostomy wound of right kidney with drainage; carcinomatous infiltration of right suprarenal; metastatic carcinoma nodule in peritoneum; secondary carcinoma and suppuration of retroperitoneal lymph glands; ascending suppurative nephritis of left kidney, acute cystitis and ureteritis; compression of inferior vena cava and left renal vein; hypertrophy of left cardiac ventricle; senile arterial sclerosis, moderate degree; atrophic pulmonary emphysema; calcified tubercle in a right peribronchial lymph gland; acute suppurative bronchitis and early hypostatic bronchopneumonia of left lower lobe; acute parenchymatous swelling of liver, and marked exostosis of the vertebral column.

Samples of the renal stones were mixed together and analyzed for me by Miss Mary E. Maver, who obtained figures indicating an average percentage composition as follows:

$Ca_3(PO_4)_2$ — 70.70; $Mg_3(PO_4)_2$, 21.35; $CaCO_3$, 7.95.

No oxalic acid, uric acid, xanthin or silicic acid was present.

This is, then, a phosphate calculus, which is an unusual type of concretion to be found in the renal pelvis. There is a current statement in the literature to the effect that renal calculi are ordinarily composed chiefly of uric acid or urates; but more recent writers have reported that calcium oxalate is usually the chief component. Rowlands [1] found that twenty-two renal stones were composed almost entirely of calcium oxalate; phosphates were commonly present, and uric acid was absent or was found only in traces. Max Kahn [2] analyzed sixteen stones and found from 29.5 to 94.7 per cent. of calcium oxalate, from 0.2 to 40.6 per cent. of uric acid, from 2.1 to 56.2 per cent. of phosphorus pentoxid, and no carbonate. Only two of these stones consisted chiefly of phosphate, and only two had much uric acid (40.6 and 23.6 per cent), while all but three had more than 70 per cent. of calcium oxalate.

Such calculi as the one in this case, composed chiefly of a mixture of calcium and magnesium phosphate, ordinarily indicate the presence of long-continued ammoniacal decomposition of the urine. Under such cases these salts may precipitate on a previously existing urate concretion, and the urates in time may dissolve out and be replaced by phosphates, constituting the process of "metamorphosis" of calculi.[3]

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1. Rowlands: Biochem. J. **3**:346, 1908.
2. Kahn, Max: Study of the Chemistry of Renal Calculi, Arch. Int. Med. **11**:91 (Jan.) 1913.
3. Wells, H. Gideon: Chemical Pathology, Ed. 4, 1920, p. 461.

The infrequency of tumors of this sort is indicated by the following statement made by Ewing [4] in his work on neoplastic diseases:

> Squamous-cell carcinoma of the pelvis was described by Kundrat in 1891 and later cases have been reported by Rundle, Graupner, Kischensky, Beisenbruch and Scheel. These remarkable tumors are usually of large size when discovered, but Battle observed squamous changes in a small villous tumor of the pelvis and Rundle found the upper ureter invaded by a squamous carcinoma of moderate dimensions. In other cases the pelvis has been distended and the wall infiltrated by a bulky growth. The kidney is either transformed into large cysts by hydronephrosis or infiltrated and destroyed. The squamous changes are very pronounced and much of the growth may be composed of epithelial pearls, many of which are hornified or calcified. In Schmorl's case, distant metastases showed the same structure. That the pelvic and ureteral epithelium is capable of extensive epidermization is well attested by the reports of Wendel of numerous cases of leukoplakia, usually associated with calculi. Rokitansky described a very advanced condition, with much scaly desquamation, as "cholesteatoma." Ziegler found marked epidermization in pyelitis calculosa, and Beselin reported an advanced case with tuberculous pyelitis. Yet all cases are not associated with calculi or leukoplakia, so that the excessive hornification must be regarded as a tendency inherent in the growth.
>
> In a case studied by the writer in a woman of 58 years, the kidney and tumor measured 14 by 19 cm. The tumor involved chiefly the extrapelvic fat but had occluded the lower half of the pelvis, along which it invaded the kidney. The renal cortex was thinned out over several large smooth-walled cysts. The ureter was destroyed down to the bladder. There were metastases in aortic and mesenteric nodes and in the uterus. The main bulk of this large tumor was composed of hornified and slightly calcified epithelial pearls surrounded by a moderate number of spindle or cubical granular epithelium. The transition from one to the other type of cell was very sharp.

Kaufmann says: "Squamous epithelial cancers occur, which sometimes follow epidermoid transformation of the mucosa, e.g., as a result of concretions. In one such case in Basle the renal pelvis contained a large branching stone (oxalate nucleus with calcium salt mantle) weighing 93.1 gm."

The case reported by Kischensky [6] resembles my case in many respects, especially in the complete replacement of the entire kidney by squamous-cell carcinoma; it also extended into the liver and diaphragm and produced metastases in the regional lymph glands and in the right knee. There was not only squamous-cell cancer tissue, but also areas of transitional and cylindric epithelial growth, and papillomatous formations. In addition to the hornification there was considerable

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4. Ewing, James: Neoplastic Diseases, a Textbook on Tumors, Ed. 1, Philadelphia, W. B. Saunders Company, 1919, pp. 743-744.

5. Kaufmann: Spezielle path. Anat., Ed. 6, **2**:877.

6. Kischensky: Ziegler's Beitr. **30**:348, 1901.

calcification. However, in this case no concretion was present. He gives a full discussion of the literature on tumors and metaplasia of the renal pelvis to that date.

While squamous-cell cancers of the kidney are rare, as a matter of fact, any type of epithelial tumor seldom arises in the pelvis of the kidney, for in 1921 Miller and Herbst [7] in reporting a case of papillary tumor of the pelvis mentioned only fifty-three others as previously reported. Metastases were observed in fifteen of these cases. Kretschmer,[8] in 1917, reviewed the literature on "Primary Non-papillary Carcinoma of the Renal Pelvis," of which he found forty-three cases, including one of his own. In eleven cases there were found, associated with the tumor, renal stones. Necropsies were secured in but nineteen cases, with metastases found most frequently in the liver (eight cases), and in five cases there were osseous metastases. He makes no particular reference to the question of hornification, and from his abstracts of the case reports it is not possible to determine this matter exactly, since the terms pavement epithelioma and epidermoidal cancer are used vaguely. Apparently only about five cases were definitely keratininzing squamous carcinomas, and in three of these renal stones were found. The cases described by Kaufmann and Ewing were not included in Kretschmer's summary. In all, I have found eleven cases of squamous-cell carcinoma of the renal pelvis sufficiently thoroughly reported to be unquestionable. In these the presence of calculi is mentioned in six.

A case reported by Primrose [9] is particularly interesting since it arose in the pelvis of a horseshoe kidney with calculous pyonephrosis. Unfortunately, necropsy was not obtained and only the observations made at operation are recorded.

Recently Scholl [10] has reported six cases of squamous-cell carcinoma of the urinary bladder, in only one of which did stone occur. In reviewing the literature on the topic he discusses also cases of squamous-cell carcinoma of the renal pelvis. He states that in 195 tumors of the kidney observed at the Mayo Clinic there was only one squamous-cell carcinoma of the pelvis of the kidney; but he does not mention whether there was or was not a stone present. This is presumably the case recorded, but not fully described, by Wilson.[11]

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7. Miller, E. M., and Herbst, R. H.: Papillary Epithelioma of the Kidney Pelvis, J. A. M. A. **76**:918 (April 2) 1921.

8. Kretschmer, H. L.: J. Urology **1**:405 (Aug.) 1917.

9. Primrose, Alexander: Squamous-Cell Carcinoma of the Kidney, J. A. M. A. **75**:12 (July 3) 1920.

10. Scholl, A. J., Jr.: Squamous-Cell Carcinoma of the Urinary Bladder, Arch. Surg. **3**:336 (Sept.) 1921.

11. Wilson, L. B.: Collected Papers of the Mayo Clinic, 1912, p 303.

Scholl also makes the following statement:

> Leber reports the case of an infant of 4 months in whom the epithelial lining of the pelvis of the kidney was composed of many layers of cells showing cornification; a similar condition was found in the infant's eye. This case suggests a congenital origin. Lecène holds that the process in these cases is due, not to chronic inflammation, but to developmental changes in the genito-urinary tract. Wilson suggests that epidermoid growths of the pelvis of the kidney may be due to inclusions of ectodermal cells carried from the rectum to the kidney by way of the cloacal wall.

The last suggestion is, of course, purely hypothetical. The frequent existence of metaplastic changes in the urinary tract from chronic irritation and the fact that chronic irritation usually precedes squamous carcinoma in the urinary tract provides adequate explanation for such cases as have been observed.

As to the frequency of squamous-cell carcinoma in the urinary bladder, Scholl quotes the statement of Geraghty that in 180 epithelial tumors of the bladder at Johns Hopkins Hospital there was but one of this type. At the Mayo Clinic, however, there were found in 333 neoplasms of the bladder the six cases of squamous-cell epithelioma which Scholl describes. Among 113 bladder tumors studied by Buerger [12] there were five squamous carcinomas.

In their review of the comparative pathology of renal tumors Slye, Holmes and Wells [13] mention only the following cases in which squamous epithelial growths have been observed in the kidneys of lower animals: "Baird [14] has reported a case of keratinizing epithelioma in the kidney of a fowl, and Borrel and Masson [15] have also described a renal tumor in a fowl which showed both cylindrical and squamous elements."

SIGNIFICANCE OF METAPLASIA

The occurrence of metaplasia in the urinary tract has been described especially often, presumably because the transitional epithelium has to undergo less alteration to produce squamous epithelium than does the columnar epithelium of the bronchi, uterus and the gallbladder, where metaplasia has been occasionally observed. Richey [16] has recently discussed the topic of metaplasia in the renal pelvis in some detail, and makes the following statement:

> That leukoplakia may be the predecessor of carcinoma is indubitable. Osler states that 20 per cent. of leukoplakia of the tongue becomes carcinomatous,

12. Buerger, L.: Surg., Gynec. & Obst. **21**:179 (Aug.) 1915.
13. Slye, Holmes and Wells: J. Cancer Res., to be published.
14. Baird, A. T.: J. Cancer Res. **2**:103 (Jan.) 1917.
15. Borrel and Masson: Bull. de l'ass. franç. p. l'étude du cancer, Paris, **4**:172, 1911.
16. Richey, DeW. G.: J. Lab. & Clin. Med. **5**:635 (July) 1920.

while Albarran, Barker, Ewing and others indicate that leukoplakia in any organ should be watched carefully for malignancy, often being, in the absence of early carcinoma, very suspicious of precancerous changes. Morris collected twenty-seven cases of tumors originating in the kidney pelvis, of which ten were malignant, while recently Kretschmer was able to gather forty-three instances of nonpapillary carcinoma of the renal pelvis, including twenty-one of apparent transitional or squamous type. The former author states that the most frequent form of carcinoma is transitional or squamous-cell, pointing out that long-standing irritation may lead to the transformation of the renal epithelium to an epidermic character, with a resultant leukoplakia which may be the starting point of squamous-cell cancer.

The formation of metaplastic squamous epithelium brings forward two puzzling topics, one chemical, the other embryologic. The chemical peculiarity is that squamous epithelium is characterized by the formation of keratin, which is a definite chemical compound, formed normally, as far as is known, only by cells of ectodermal origin, including the neurokeratin of the central nervous system. When cells of endodermal origin, such as those lining the gallbladder, or those of mesodermal origin, such as those lining the renal pelvis or the uterus, take on the function of forming this peculiar, insoluble, sulphur-rich, indigestible protective chemical, keratin, they have assumed a chemical function which seems to be far removed from their normal capacity. Hence we must conclude that metaplasia involves not only a morphologic but a chemical transformation in the cells.

For tumor pathology another problem arises. When cells assume the proliferative activity that is characteristic of malignancy, they usually lose their more recently acquired functions and retain chiefly the simple vegetative function of proliferation. But when a transitional or columnar epithelial surface becomes squamous through metaplasia, and the same protracted irritation that produced the metaplasia continues until cancer results, we find that the newly acquired property of forming keratin has become fixed and the cancer is a keratinizing, squamous-cell carcinoma. One would expect the epithelium to approach its original, simpler embryonal character, rather than exhibit and retain so profound and recently acquired an alteration as the production of keratin.

SUMMARY

The formation of keratinizing squamous-cell carcinoma in the renal pelvis is a rare occurrence. In the case reported, which is of this sort, the metaplasia of the transitional epithelium to the squamous form was apparently the result of chronic irritation from renal concretions.

Intracranial Aneurysm of the Vertebral Artery

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INTRACRANIAL ANEURYSM OF THE VERTEBRAL ARTERY *

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CHICAGO

Although small aneurysmal dilatations are common in any and all of the cerebral vessels, large intracranial aneurysms are rare. Thus, Hoffmann,[1] in reporting on seventy-eight intracranial aneurysms observed during twenty years' examination of medicolegal cases in Vienna, describes chiefly minute dilatations or diffuse enlargement of the cerebral arteries, and not aneurysms of a size that would be likely to produce pressure symptoms. Most of these cases had come to necropsy because of sudden death from rupture of the dilatations, and had not been observed clinically. The aneurysms were distributed as follows in seventy-five cases:

Arteries of the sylvian fissures	20
Carotids	13
Anterior communicating	12
Basilar	10
Vertebral	10
Miscellaneous	10
Total	75

Of these, fifty-three were in women and twenty-two in men, which reverses the proportions usually observed in aneurysms. It is to be noted that of the ten aneurysms of the vertebral artery, seven were on the left side; this predominance of the left side is usually described. Thus, Oppe,[2] who in 1892 collected reports of seven cases of aneursyms of the vertebral artery, found that six were in the left.

Möser,[3] describing three cases of what were virtually simple dilatations of the left vertebral artery, comments on the fact that thrombosis and embolism also usually affect the left vertebral artery. He made a study of the vertebral arteries in several bodies and found that the left artery is usually somewhat larger than the right (average, 2.43 as against 2.18 mm. diameter), but that there is almost always a constriction at the point where the left vertebral joins the basilar, and less often on the right side, the average amount of narrowing being 0.318 and 0.24 mm. for the left and right arteries, respectively.

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* From the Department of Pathology, University of Chicago, and the Otho S. A. Sprague Memorial Institute.

1. Wien. klin. Wchnschr. **7**:824, 1894.
2. München. med. Abhandl. Ser. 2, 1892; quoted by Steckelmacher.
3. Möser: Deutsch. Arch. klin. Med. **35**:418, 1884.

In 142 cases of cerebral aneurysm reviewed by Krey [4] none was in the vertebral arteries. Beadles [5] compiled 555 cases of aneurysm of the cerebral arteries, all but 114 of which had been previously published. Unfortunately, he gives no analysis of this great material, but he has little to say concerning vertebral artery aneurysms, so presumably they were not numerous. He mentions only three such cases. The chief facts brought out by him are that in 46.3 per cent. of cerebral aneurysms the first symptom was apoplexy, in 67 per cent. death was preceded by apoplexy, and in 20.7 per cent. there were clinical evidences of intracranial tumor, which most often occurred with aneurysms of the basilar artery, which he discusses at some length. In only 16.39 per cent. was there no cerebral or meningeal hemorrhage. He describes one aneurysm of the left internal carotid artery which measured 1¾ by 1⅛ (45 cm. by 29 cm.) inches, and states that this ranks among the largest intracranial aneurysms on record. "Its size has been exceeded by few, probably less than a dozen." These large aneurysms have arisen mostly from either the carotid or the basilar artery; he makes no mention of vertebral aneurysms in this group. Some of these aneurysms were the size of "a small orange," "a duck's egg," "a small hen's egg," "the size of a nut," whatever these comparisons may signify. Some, with measurements given, exceeded 2 inches in diameter. The largest vertebral aneurysm that I have found reported, that of Steckelmacher,[6] is uncertain since the diagnosis rested on clinical evidence only. The aneurysm eroded the left half of the supra-occipital bone, and formed a large visible external swelling. The patient was still alive when the case was reported, so the origin cannot be considered as positively established.

The classical illustration of aneurysm of the vertebral artery published by Cruveilhier [7] in his famous atlas, involved the right vertebral artery, and, to judge from the illustration, measured about 15 mm. in diameter.

There has appeared recently a report of a case of vertebral aneurysm of comparatively large size, described by J. Floyd Morrow.[8] This was observed in a body used for dissection in a medical school, and consequently there was no history.

The man was apparently 50 years of age, and, as no other evident cause for his death was found, it was assumed that he had died from paralysis of

4. Inaug. Dissert. Greiswald quoted by Rhein: J. Nerv. & Ment. Dis. **41**:360, 1914.

5. Beadles: Brain **30**:285, 1907.

6. Steckelmacher: Deutsch. Ztschr. Nervenheilk. **54**:347, 1916.

7. Cruveilhier: Anatomie Pathologique du Corps Humain **2**:28, 1835, pl. 3.

8. Morrow, J. Floyd: Med. Rec. **100**:894, 1921.

the vital centers. The aneurysm is described as "a spontaneously cured fusiform aneurysm of the right vertebral artery. The aneurysmal dilatation began 1.5 cm. from the bifurcation of the basilar artery, and increased abruptly to its greatest diameter of 2.5 cm. This extreme dilatation was continued for a distance of 3 cm. downward and the artery again very abruptly assumed its normal diameter." There was a channel on the side toward the medulla, of approximately normal size. There was much calcification of the wall. The left vertebral artery was crowded laterally and showed a dilatation for about 2.5 cm. of its course with a diameter of nearly 1 cm. This artery was very sclerotic as also was the circle of Willis. The medulla was pressed into an unrecognizable mass by the aneurysm. The anterior surface of the medulla was concave, much flattened and pushed backward between the cerebellar lobes, but there was no softening. The pons was pressed slightly upward on the left. Much degeneration of sensory and motor tracts was shown by Marchi staining. There is no statement made as to evidence of syphilis elsewhere in the body.

Most of the other cases recorded concern small aneurysms, like the "cherry pit" sized aneurysm of the left vertebral artery reported by Schultze,[9] which was unusual in producing a marked convulsive tic involving the left facial nerve, without degeneration of the nerve. In this case death resulted from pulmonary tuberculosis. We also have the two vertebral aneurysms observed among twenty-two cases of ruptured intracranial aneurysms described by Wichern.[10] Both of these were in males, one in the right vertebral artery, 3 to 4 mm. in diameter, and one in the left, "a pea sized sac."

Weidman [11] states that since Beadle's report in 1907 only seven cases have been indexed up to 1915. The largest of these aneurysms was reported by Boinet,[12] measuring 30 by 15 mm., involving the left vertebral artery, and associated with several gummas of the brain and meninges. An aneurysm like that in Wiedman's case also occurred in a man, aged 40, with syphilis, but it was only "pea sized."

A striking feature about most of the ruptured cases, however, is the lack of any mention of the presence of evidences of syphilis. Usually the absence of such evidences is particularly mentioned.

Turnbull [13] discusses the etiology of forty-eight cerebral aneurysms and finds that none of them seemed to be of syphilitic origin, although he recognizes that this is the dominant cause of aneurysm of the aorta, and occasionally in other arteries. In this series he attributed fifteen aneurysms to infected emboli and thirty-three to medial degeneration

9. Virchows Arch. f. path. Anat. **65**:385, 1875.

10. Wichern: Deutsch. Ztschr. f. Nervenh. **44**:220, 1912.

11. Weidman, Fred D.: Ruptured Aneurysm of the Right Vertebral Artery, J. A. M. A. **65**:1105 (Sept. 25) 1915; Am. J. M. Sc. **151**:427, 1916.

12. Compt. end. Soc. de Biol. **69**:210, 1910.

13. Turnbull: Brain **41**:50, 1918.

following congenital developmental deficiency. Hedinger[14] reports an aneurysm (1 cm. by 4 to 5 mm.) in the left vertebral artery observed shortly after a fall backward downstairs with injury to the neck and shoulders, and believes that the aneurysm was of traumatic origin since there were no evidences of arterial disease elsewhere, and no signs of syphilis. He speaks of having had also three cases of ruptured aneurysm of the "arteria vertebralis et basilaris" in syphilitic patients, without giving details.

Several other writers on intracranial aneurysm have attributed importance to traumatism in their etiology. It is indeed easy to imagine that the larger arteries may readily suffer traumatism from violent blows on the skull, since we know from the evidence concerning fractures and cerebral contusions *a contre-coup,* that sudden alterations in intracranial relations take place that must almost certainly put a sudden severe strain on the large vessels at the base on account of their fixation to the skull.

Direct traumatic aneurysms in the cervical portion of the vertebral artery have been described, especially from wounds received in the World War, but these do not concern us here. The literature has been largely collected by Kuttner[15] and Kausch.[16]

The evident rarity of large intracranial aneurysms in the vertebral arteries makes it desirable to put on record a case that I have recently examined, despite the fact that no adequate neurologic examination was made before death. This is especially regrettable since the size and location of the aneurysm were such that symptoms of a cerebellopontile angle tumor should certainly have been present, and we know that some at least were.

REPORT OF A CASE

The subject was a colored man, 54 years old, who entered the Illinois Central Hospital on Dec. 21, 1920, complaining of pain in the left side, loss of weight and weakness. He had difficulty in urination; the urine was scanty, and the patient had had nocturia during the last three months, getting up two or three times during the night and passing only a small amount of urine. He had had some dizziness since September, during which month it was marked. He had also diminished vision.

The patient gave a history of typhoid, pneumonia and measles during childhood, and malaria twenty-two years ago. He used whisky slightly and admitted having had gonorrhea but no chancre. His wife was living and well; she had not had children, but had an abortion soon after marriage. Two of his brothers were dead, and he had four sisters who were living and well. The man had been ill for about four months; he became ill in bed in September and had worked two weeks since that time.

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14. Hedinger: Cor.-Bl. f. schweiz. Aerzte **47**:1393, 1917.
15. Kuttner: Beitr. klin. Chir. **108**:1, 1917.
16. Zentralbl. f. Chir. **45**:885, 1918.

Physical Examination (made in the hospital).—The pupils reacted to light and accommodation and were regular. The patient expectorated large quantities of whitish yellow sputum having a foul odor. The lungs showed dulness over both bases. Mucous râles were present over both sides anteriorly. Several teeth were missing and one was carious. The tongue was furred. The tonsils were hidden. No paralyses of any kind were noted.

The apex beat was under the nipple at the fourth interspace. The left border of the heart was 2 cm. outside the nipple. The right side was under the sternum. The heart sounds were normal.

The abdomen was flat, contained no fluid, showed no tenderness and no organs were palpable. The genitals were normal. There was a slight inguinal adenopathy. The knee reflex was absent, the patient being moribund.

Roentgen-ray examination showed pyorrhea of all the remaining teeth. The heart was greatly enlarged, lying in a transverse position, greatly distended and pulsating heavily. The urine showed a few pus cells and hyalin casts. While in the hospital the patient coughed a great deal, especially when trying to swallow liquids, which he swallowed with much difficulty.

Course of Illness.—On January 1, the patient fell out of bed. On January 2, he was unable to take food and coughed a great deal; his breathing was labored; he still swallowed with difficulty. On January 4 he was semiconscious, the pulse and the general condition were poor.

The temperature fluctuated between 101 and 103 F. until the last day when it rose from 99 to 106. The pulse increased from 100 on the day of admission to 160 on the day of death, January 5.

These findings merely indicated a bilateral pneumonia, probably hypostatic, and in the advanced stage of this terminal infection, with the patient moribund most of the time, no evidence of nerve lesions was detected. Evidently there were no marked facial or ocular paralyses, since doubtless these would have been observed. After the necropsy the widow was interviewed, and we obtained the following additional facts:

History Obtained from Widow.—When the patient was about 18 years old, while walking on a railroad trestle, he was struck by a train. He was knocked to the ground about 12 or 15 feet (365.76 to 457.2 cm.) below, and his forehead hit a rock. He remained unconscious for three or four days. The injury on his forehead was described as being about "the size of a fifty-cent piece." He was treated at home and no surgical procedures were employed. As far as could be ascertained, there was no immediate functional effect, but the patient had said that he had felt foolish for some time afterward. He was married when 23. No history was obtainable of his condition in the interval between injury and marriage. He was apparently well until eight or ten years ago when he began complaining of headaches. At times he would sit and hold his head in his hands because of the pain. He complained particularly of pain at the top of his head where it felt "as if the flesh was nailed to his head." These headaches became worse. He worked as an elevator man for the last five years before his death.

About three years ago, while sitting in a chair, the patient had what his wife called a "spell." He shook all over for about ten or fifteen minutes, after which he had a high fever and sweated profusely, and said his eyes felt as if they were crossed. A physician was called, who advised him to stay in bed. He remained in bed five days and at home twelve days. His body was tender all over; he could not stand to be rubbed with a liniment. A physician

prescribed mercury for him. He went back to work but was very weak for one week. A few months later in the same year he had another similar attack. During the attack his mind was clear. He went to work the next morning but was weak. Two months later he had a third attack and went to work the next morning but was weak the day following.

The following autumn he complained of his teeth and was advised to have them extracted. About three months later two teeth were extracted. A few months afterward he came home sick, still complaining of his teeth. He was advised by the company physician to have more teeth extracted, which was done, but no relief was obtained. He felt as if he were cross-eyed. He was fitted with two pairs of eyeglasses but obtained no relief. He was always complaining of roaring in his ears. After having the teeth extracted he went to work. One day in November, 1920, while at work, he had what was called a paralytic stroke. He fell down in the elevator and had to be assisted to his feet. Information was not obtained as to whether he became unconscious or not. He recovered from this attack and came home alone, but later found he could not walk steadily. About three weeks later he complained that his legs felt like lead although he was able to move them slightly. He lost control of urination and stools, became unable to articulate and could not swallow. He grew steadily worse. His eyes were affected so that he could not recognize objects held before them. Apparently no facial paralysis existed. He was then taken to the hospital.

The records of the company physician contain no reference to any symptoms that could be referred to the intracranial tumor, and physicians who saw him daily at his work observed no evidence of any facial paralysis.

Necropsy Examination.—This examination was performed about eighteen hours after death.

External Appearance: The body was that of a well nourished and well developed colored man about 54 years old. There was no icterus. The superficial lymph glands were not noticeably enlarged. There was no edema of the ankles. The edges of the tibiae were smooth and there were no scars on the external genitals. There was about 1 cm. of subcutaneous fat. A small fatty hernia of the round ligament of the liver was found in the midline, about 2 cm. in diameter.

Pleural Cavities: The left pleural cavity was free from fluid and from adhesions except between the pericardium and the base of the left upper lobe. The thymus was replaced by fat.

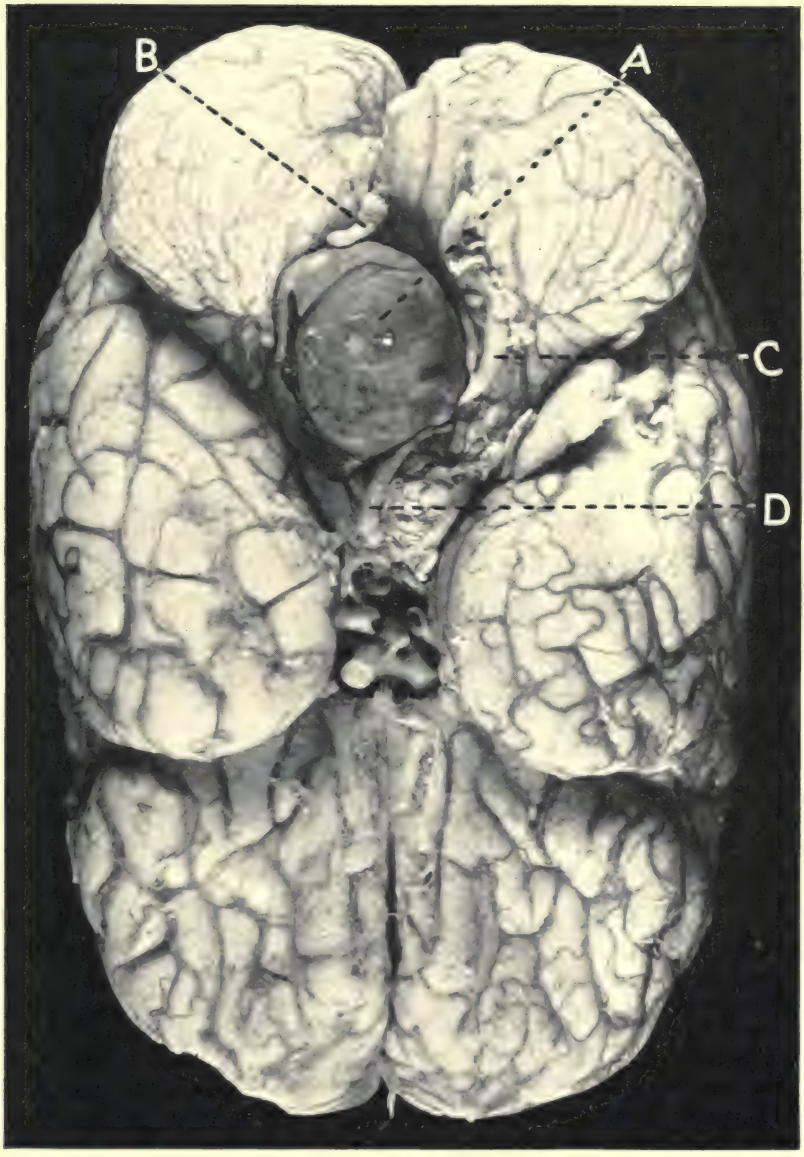
The right pleural cavity contained a few adhesions along the anterior margin of the lower lobe. Elsewhere it was free from adhesions and contained no fluids.

Pericardial Cavity: This was normal.

Mouth and Pharynx: Many teeth were missing, and there was infection of the gums around those remaining. Otherwise there were no changes.

Larynx and Trachea: These showed no changes.

Heart, Aorta and Vessels: The heart was considerably enlarged, chiefly by hypertrophy of the left ventricle. The coronary vessels from the outside showed many calcified spots. The inner surface of the coronary arteries showed numerous yellow sclerotic patches. The valves were normal. There were extensive subendocardial hemorrhages in the left ventricle, chiefly in the upper portion. The myocardium was apparently normal except for a few small areas of fibrosis. The wall of the left ventricle was 15 mm., of the right ventricle



A, aneurysm of the left vertebral artery. Note that it has crowded and compressed the medulla upward and to the right. The defect observed was produced by removing material for sections. *B*, left vertebral artery which abruptly enters the aneurysmal sac; *C*, right vertebral artery; very sclerotic and crowded to the right; *D*, basilar artery.

3 to 4 mm., thick. There were a few small sclerotic patches in the arch and descending portion of the aorta. Both cavities of the heart were enlarged. The estimated weight of the organ was 400 gm.

Lungs: These showed marked bilateral hypostatic congestion with bronchopneumonia. The peribronchial lymph glands were extremely anthracotic but showed no evidences of caseation or calcification.

Liver and Gallbladder: These organs were normal.

Spleen: The spleen was small and distinctly fibrotic but not hard. There were fibrous adhesions between the spleen and the stomach.

Pancreas: The pancreas was small but normal in appearance.

Gastro-Intestinal Tract: The stomach showed numerous submucous hemorrhages, especially in the cardia, but was otherwise normal. The intestines showed no abnormality.

Suprarenals: The suprarenals were normal.

Kidneys: The kidneys were slightly smaller than usual. The cut surface of the left kidney showed the cortical markings well preserved. The capsule stripped readily leaving a smooth surface. The right kidney was also normal.

Urinary Tract: The urinary bladder was normal.

The prostate was somewhat enlarged. Nodules of hyperplasia were present in the median lobe, which protruded slightly under the mucosa of the bladder.

The seminal vesicles were normal.

There were numerous calcified thrombi in the periprostatic veins.

Lymph Glands: In general, these were normal.

Brain and Meninges: There was some rachitic deformity of the skull. The dura was very thin. The veins on the surface were distended. The vertex of the brain was in close contact with the dura. There was a small amount of clear cerebrospinal fluid. The sulci of the brain were much flattened, and the lateral ventricles were greatly dilated, each measuring 25 mm. across in the widest parts. The hypophysis was normal. There was a nearly spherical, chocolate-brown tumor lying exactly at the junction of the pons and the medulla, both of which were compressed (see illustration). The tumor was nowhere adherent but was held in place by the blood vessels which entered it. About 80 per cent. of the tumor lay to the left of the median line. The basilar artery was crowded to the right and the left vertebral artery entered the tumor at a point near the inferior external angle. This artery reappeared again at a point 2 cm. nearer the midline at the same level, at a point 7 mm. from its junction with the basilar artery. The covering of the mass was continuous with that of the artery. The mass measured 34 mm. in its anteroposterior diameter, 30 mm. transversely, and 35 mm. vertically. The superior surface forced itself into the space formed by crowding the medulla from the cerebellum, reaching a point about 15 mm. above the inferior surface of the medulla. It was filled with a mass of dark, granular clot, somewhat laminated, with numerous calcified areas near the arterial side, and no fluid blood. The shape of the aneurysm had been partly molded by the adjacent brain tissue, for there was a somewhat pyramidal projection of the superior surface into the angle between the cerebellum, medulla and pons. The basilar and vertebral arteries were very sclerotic with areas of calcification, and the other cerebral arteries also were sclerotic.

The left vertebral artery had an extremely thick wall in the portion proximal to the aneurysm, with only a minute lumen remaining. Distal to the aneurysm it was occluded by a thrombus up to the point where it entered the basilar

artery. The left vertebral artery was only about one half the size of the right, which was of the same diameter as the basilar artery. Apparently the left vertebral artery had been occluded for a long enough time to permit secondary sclerosis of the left and compensatory dilatation of the right vertebral artery. There were a few soft protrusions from the surface of the tumor which was covered by a smooth capsule. The cerebellum was slightly compressed at the anteromesial angle, and the medulla was pushed upward and to the right, being distinctly curved. The sixth to ninth left cranial nerves were flattened between the aneurysm and the cerebellum. The anterior margin of the tumor was just posterior to the fifth nerve origin.

There was a distinct grooving at the base of the skull involving the sphenoid bone and the occipital bone at the site where the tumor lay, just anterior to the foramen magnum. The dura was intact at this place, and the tumor was not adherent to it, nor was the dura abnormally adherent to the skull at this point. No evidence of any old injury to the skull was observed.

Microscopic examination of the wall of the aneurysm showed it to be composed solely of a thin membrane of dense hyalinized fibrous tissue, in which no elastic tissue or other remains of the artery wall could be detected, at least in the portion examined. The sac contained masses of clotted blood, much disintegrated, with no evidence of infection. There were a few small calcified masses attached to the vessel wall but within the clot. There was no evidence of organization of the clot, and no inflammatory reaction in the wall of the aneurysm.

The aorta showed merely a simple senile sclerosis. Neither here nor elsewhere could evidence of syphilis be found. No microscopic changes of importance were found in the other tissues.

Anatomic Diagnosis.—The anatomic diagnosis was: Saccular aneurysm of the left vertebral artery, with compression of the pons, cerebellum and medulla, and roots of the sixth to the ninth cranial nerves; pressure deformity of base of the skull; dilatation of the cerebral ventricles; bilateral hypostatic bronchopneumonia; diffuse senile arteriosclerosis; hypertrophy of the left cardiac ventricle; slight fibrous myocarditis; ventral fatty hernia; slight rachitic deformity of the skull and tibiae; subendocardial hemorrhages.

SUMMARY

A colored man, with no clinical or anatomic evidence of syphilis, but with a history of severe cranial traumatism many years before, died of hypostatic bronchopneumonia, without giving marked evidence of increased intracranial pressure or of cranial nerve paralysis during his last illness. Necropsy examination revealed a large aneurysm of the left vertebral artery, causing much deformity about the cerebellopontile angle, compressing the sixth to ninth cranial nerves, occluding the left vertebral artery, and, by pressure on the aqueduct of Sylvius, causing distinct internal hydrocephalus. That the swelling had been present for a long time was indicated by the presence of a distinct grooving in the floor of the skull beneath it. A necropsy inquiry revealed that the patient had had roaring in the ears, dizziness and "spells" three years before his death. Neuralgic pain and infection about the gums led

to the extraction of several teeth four months before death. Two months later there was an attack of syncope. Three weeks before death difficulty of speech, decreased vision and difficulty in swallowing appeared, terminating in hypostatic bronchopneumonia.

As far as can be learned from the literature, this seems to be one of the largest vertebral aneurysms yet observed. It is unfortunate that the patient had not been the subject of a thorough neurologic examination during life, for the differentiation from a cerebellopontile angle tumor would have afforded difficulty. In two or three recorded cases a bruit has been heard over a vertebral aneurysm, but the clue would not have been present here since the artery was completely occluded. This aneurysm corresponds to the statement in the literature that intracranial aneurysms are not usually the result of syphilitic arteriosclerosis, but that they often give a history of antecedent cranial trauma. The presence of a distinct grooving of the skull beneath the aneurysm, without adhesion or erosion, would support the hypothesis that the aneurysm might have formed after the injury to the 18-year-old boy. Probably this yielding of the skull permitted the aneurysm to reach the exceptionally large size it had attained.

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THE PATHOLOGY OF BRONCHIAL ASTHMA*

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INTRODUCTION

In a clinical study of a certain type of food asthma, published nearly ten years ago,[1] one of us expressed the conception that bronchial asthma is a manifestation of allergy in the human, in the following concluding sentences: "In most cases of bronchial asthma the causative factor lies in the allergic reactibility of the individual," and, "The multiplicity of asthmatogenous substances is explained by the multiplicity of proteins which may act as anaphylactogens."

The later clinical and immunologic studies of Walker, Cooke, Rackeman, and Mackenzie, undertaken from the same point of view, have greatly elaborated the evidence for this conception and have brought forward more detailed proofs for its correctness and general validity for the majority of all cases of asthma. Since allergy in the end means changed tissue reaction, it is obvious that the investigation of any allergic condition in the human must necessarily take up the question whether demonstrable morphologic changes are associated with the physicochemical changes which are the first reactions of the organism toward the protein acting as an allergen.

The histologic examination of the animal body postmortem has furnished, in one particular form of allergy, anaphylaxis, very important information which is of the greatest value in the diagnosis and characterization of the anaphylactic death and for our understanding of the pathologic physiology of this immunologic phenomenon. It suggests at the same time that different organ-systems and structures are primarily involved in different species of animals, and opens the still unanswered question whether all tissues of the same species are allergic to the same extent.

Up to the time of the allergic conception of bronchial asthma, the students of this disease, neglecting too much the true underlying condition, had focused their chief attention on the respiratory crisis, the paroxysmal attacks of dyspnea. This symptom fascinated the

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1. Koessler, K. K.: Bronchial Asthma Due to Hypersusceptibility to Hen's Eggs, Illinois M. J. **23**:66, 1913.

physician on account of its explosive and dramatic character and, because of the intense discomfort of the patient, demanded immediate relief, often taxing the resources of medical aid to the utmost. While the respiratory crisis of asthma comprises in itself no more of the pathologic physiology and true nature of the disease than do the convulsive attacks in eclampsia or epilepsy, it has developed in this way that most discussions of this condition have dwelt chiefly on the origin and mechanism of the dyspnea.

The theories that have been formulated to explain the attacks of paroxysmal dyspnea are agreed today that the difficulty of respiration is due to a stenosis of the bronchi, but whether this narrowing is due chiefly or exclusively to a spasm of the bronchial smooth muscle system, to swelling and exudation of the bronchial mucosa, to a true obturation stenosis by the secretion from the bronchial glands, or to a combination of two or more of these conditions seems still to be an unsettled question. The recognition that certain forms of bronchial asthma are manifestations of allergy in man, a conception which has introduced a new era in the study and treatment of this disease, has strengthened the contention that bronchiolar spasm is the important factor in the production of the bronchostenosis. It must be admitted, however, that this view, aside from the indirect proof obtained from the pharmacologic action of atropin and epinephrin, has mainly been deduced from the mental comparison and identification of the emphysema and bronchospasm observed in the classical experiment of anaphylaxis in the guinea-pig and not from a detailed study of the lung structures of the human asthmatic after death.[2]

The diffuse and loose application of the term "anaphylaxis" to every kind of diurnal or nocturnal dyspnea will not advance our knowledge of bronchial asthma. Anaphylaxis is a well defined immunologic phenomenon, and the application of this term should be restricted to those forms of asthma which fulfil at least some of the following conditions:

1. Positive skin sensitization tests for one or more specific proteins. It is possible, however, to be sensitized in the allergic sense and to show no skin sensitization.[3]

2. Blood, sputum and tissue eosinophilia, showing an altered reaction of the human organism to a foreign soluble protein.

2. Meltzer, S. J.: Bronchial Asthma as a Phenomenon of Anaphylaxis, J. A. M. A. **55**:1021 (May 9) 1910.

3. Fleischner, E. C.; Meyer, K. F., and Shaw, E. B.: A Résumé of Some Experimental Studies on Cutaneous Hypersensitiveness, Am. J. Dis. Child. **18**:577 (Dec.) 1919.

3. Relief from the bronchial spasm by the use of atropin or epinephrin.

4. Desensitization of the patient by repeated injections of the exciting protein, e. g., horse dander, pollen or by ingestion of gradually increasing quantities of the exciting protein, e. g., egg-white.

5. Freedom from symptoms on removal of the exciting protein.

6. Passive sensitization of a laboratory animal with the blood of the asthmatic.

7. Postmortem examination in fatal cases to determine if there are any changes which account for the sudden death and which simulate the acute emphysema found post mortem in the guinea-pig and to exclude the dyspnea due to capillary thrombosis or embolism (anaphylactoid reaction of Karsner and Hanzlik); for it is chiefly the pathologic-anatomic examination of the animals postmortem that has characterized the anaphylactic symptom complex peculiar for each species.

On the other hand, we believe it to be a grave mistake to consider all cases of bronchial asthma as a manifestation of allergy. The fallacy becomes especially apparent in a study of the bacterial type of asthma, the so-called asthmatic bronchitis.[4] Bacterial infection may lead in a variety of ways to bronchial stenosis. The infection of the mucosa and the glandular structures may produce an abundant exudate leading to partial or total obstruction of certain bronchi, or the infection may lead to profound injuries of the protecting layer of the mucosa, causing minor losses of substance, thus facilitating absorption of toxic materials which stimulate muscular spasms similar in mechanism to the bronchiolar spasm produced through inhalation of excoriating gases. It is also not improbable that various poisons of the type of peptones or amines are formed by the action of bacteria on tissue proteins. These poisons, when carried by the blood or lymph stream from the intestinal tract, or from distant foci of infection to the peripheral nerve endings in the bronchial musculature, may stimulate it to spastic contraction. Only in very rare instances might bacterial proteins, as such, sensitize a person and act as anaphylactogens. Then the bacterial type of asthma, too, might become allergic in character. Even in the instances of allergic asthma a chronic bronchial infection is often superimposed as a second pathogenetic factor, which only too often obscures, especially in older persons, the primary allergic susceptibility of the patient.

4. Koessler, K. K., and Moody, A. M.: Etiology of Chronic Bronchitis, with Special Consideration of Those Forms Associated with Bronchial Asthma, J. A. M. A. **64**:1104 (March 27) 1915.

These considerations suggest that detailed histological examinations of patients who have died from asthma should furnish information of value for settling certain phases of the problem briefly discussed in the preceding paragraphs.

HISTORICAL REVIEW

Since our work is mainly concerned with the microscopic analysis of the finer lung structure in bronchial asthma, we shall review here only those cases in literature in which a microscopic examination has been made. While the medical literature of the seventeenth, eighteenth and nineteenth centuries contains some excellent post-mortem reports on the macroscopic examination of persons who have died of asthma, the survey of these reports does not, at this time, warrant their inclusion here for the sake of completeness. The number of recorded microscopic studies of the lung structure of persons who, during life, have suffered from true bronchial asthma, is exceedingly small if one considers the frequency of the disorder. So far as we know, only fifteen cases are reported in which a more or less detailed microscopic study of the lungs has been made and of this number only about one-half seem, on close analysis of the data, to be cases of true bronchial asthma.

SUMMARY OF CASES IN LITERATURE

CASE 1. 1886. E. v. Leyden.[5]—*History.*—Female, aged 40, had had asthma

NECROPSY REPORT: *Macroscopic Examination.*—Both lungs show marked vesicular emphysema and their borders are greatly distended. The bronchi are not dilated and not changed, except for a reddening of the mucosa. The small bronchi contain greenish or whitish mucous masses in which there are no fibrin or crystals.

Microscopic Examination.—Some of the alveoli are distended, while others are not, and most are filled by a granular material containing many large cells. The walls of the smaller bronchi are not essentially changed and the lumen of many is practically occluded by a layer of amorphous material which is adherent to the epithelium. One illustrated section is described.

Comment.—From the history, this case appears to be a typical case of bronchial asthma which was complicated during the last months of life by failing heart and kidneys. The occlusion of many bronchi by mucus is the most significant pathologic change described.

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5. v. Leyden, E.: Ueber Bronchial Asthma, Deutsch. Militärärztl. Ztschr. **15**:51, 1886; Ueber Bronchial Asthma, Berlin, 1886, Mittler and Son. since early childhood and was treated by v. Leyden seven years previously. Her attacks appeared and disappeared suddenly, the frequency, severity and number varying considerably. In attempting to relieve her suffering she became a high grade morphin addict. Later severe hydrops and albuminuria developed. The lungs were emphysematous and sibilant râles were diffuse. During attacks, a tough grayish sputum containing fibrinous threads, small firm masses, crystals and epithelial cells were raised. Death followed an attack of severe dyspnea with cyanosis.

Case 2. 1889. J. B. Berkart.[6] *History.*—Mrs. C., aged 37; family history of tuberculosis. Childhood diseases: whooping cough, measles and scarlet fever. Since childhood she had been subject to frequent colds, especially in early summer. The first attack of bronchial asthma followed a severe prolonged bronchitis at the age of 23. Following this, she had frequent attacks of bronchitis with asthma which, with a swelling of the legs, caused her to enter the hospital a few months before death. On entrance, the heart's action was disturbed and the urine contained albumin. She left the hospital after a stay of three or four months, being considerably improved, except for a generalized edema and frequent severe attacks of paroxysmal dyspnea. Nine days before death she was seen again because of dyspnea, cough, insomnia, diarrhea and weakness. The face was cyanotic and edematous. The chest showed bilateral high pitched resonance with diffuse sonorous and sibilant ronchi. Pulse 100, small, soft and regular. Heart extended beyond the right sternal border and its sounds were loud and clear. Abdomen distended. Urine negative. Twenty-four hour specimen of sputum about 4 ounces, contained Curshmann's spirals, Charcot-Leyden's crystals and eosinophils. Death followed an acute exacerbation of all her symptoms.

Necropsy Report: *Macroscopic Examination.*—Heart, considerable dilatation and hypertrophy, with valves intact. Lungs not collapsed. Extensive adhesions of the right apex. The right main bronchus and one branch of the left main bronchus are almost occluded by dark brown cylindrical branching masses. The smaller bronchi are dilated and some are partially obstructed by exudate. The rest of the lungs are emphysematous. The abdomen contains a moderate amount of fluid. The liver, spleen and kidneys are large, pale and dry.

Microscopic Examination.—The cylindrical masses are composed chiefly of deformed degenerating cylindrical epithelium with no Charcot-Leyden's crystals. The mucous membrane of the right main bronchus shows firm, fibrous avascular excrescences covered by a single layer of oblong epithelium, almost all the remainder of the mucosa being denuded. One deep ulceration in a main bronchus extends almost to the cartilage. There are a few colonies of staphylococci between the folds of the internal fibrous layer. The mucous glands are remarkably few in number, very small, and looking as if atrophied. The bronchi of medium size are irregularly dilated, most of them partially or completely occluded by masses of detritus containing fragments of Charcot-Leyden's crystals. The walls are thickened and there is considerable hyperplasia of the mucous membrane. The finest bronchi are dilated and denuded of epithelium and some contain fibrinous threads and colonies of streptococci. The alveoli are emphysematous and have no epithelium, while some are almost filled with a fibrinous exudate.

Comment.—The clinical history of the case points to a bronchial asthma developing from a chronic bronchitis and complicated in the last few months by a failing heart. The most significant pathology is the ulceration in one of the chief bronchi, the atrophy of the mucous glands and the hyperplasia of the mucosa of the middle sized bronchi.

Case 3. 1892. A. Schmidt.[7] *History.* J. S., waitress, aged 49; previous illness articular rheumatism at 44. Six months before death, dyspnea, emaciation and weakness caused the patient to stop work. Two months before death a troublesome cough, which was relieved by raising a small amount of

6. Berkart, J. B.: Bronchial Asthma, London, 1889.

7. Schmidt, A.: Beiträge zur Kenntniss des Sputums insbesondere des Asthmatischen und zur Pathologie des Asthma bronchiale, Ztschr. f. klin. Med. **20**:476, 1892.

tenacious mucus, developed and she was unsuccessfully treated for bronchitis. About three weeks before death the first typical attack of asthma came on and was relieved an hour later by raising some tenacious mucus. Following this, attacks came on frequently and she entered the hospital. Examination showed an emaciated female with a cystic growth the size of a goose egg in the left side of the neck. The lung borders were lowered and many dry râles were heard on expiration. Severe attacks came two to three times daily and three days after entering the hospital she died in collapse following a severe attack of dyspnea.

Necropsy Report: *Macroscopic Examination.*—Tumor nodules press against and partially encircle the structures in the left side of the neck, occlude the thoracic duct and extend into the mediastinum and into the upper lobes of both lungs. A small tumor mass presses against the left pulmonary vein and partially occludes it. The heart is large and shows a marked fatty degeneration. The right ventricle is dilated. The aorta is markedly sclerotic. The apices of both lungs are adherent to the thoracic wall. The section of both lungs shows numerous tumor nodules in the upper lobes, some of which at the hilum press on the bronchi and cause narrowing of their lumina and degeneration of their walls. Peripheral to the narrowed places, the bronchi are dilated. The bronchi of both upper lobes contain yellowish tenacious mucous masses which can be pressed out or pulled out as long spirals, several centimeters in length. Both lower lobes contain much blood and exude a purulent fluid. Neither contain tumor nodules except at the hilum. The remaining organs show no important change.

Microscopic Examination.—The epithelium of the dilated bronchioles is almost intact, the lumen almost or completely occluded by mucous material which stains more deeply in the central portions and contains numerous cells, among which are alveolar epithelium, a few polymorphonuclear neutrophil leukocytes and some homogeneous round, oval or elongated structures without nuclei. The mucus in the bronchioli respiratorii shows the spiral arrangement but the alveoli contain no spirals. The mucous glands of the bronchi are in a state of active secretion, but the gland ducts contain no spirals. The walls of the bronchi and bronchioles contain dense connective tissue fibers and are infiltrated by round cells. Surrounding a few of the bronchi, which contain polymorphonuclear leukocytes, the alveoli contain numerous leukocytes of the same type. The blood vessels of the upper lobes show a marked periarteritis obliterans. The masses of tumor tissue have the arrangement of a medullary carcinoma.

The author discusses the occurrence of Curshmann's spirals, Charcot-Leyden's crystals, fibrin and eosinophil cells in the sputum and shows that, as these structures are found in so many other disorders, the sputum findings are of no great diagnostic importance in bronchial asthma. He considers the attacks of dyspnea as the all important symptom, but believes that there is no sharp distinction between the dyspneas of asthma and of severe bronchitis. But, he says, the work of Biermer and others has shown that there is a distinct nervous form of asthma in which the inflammatory factor is insignificant and he considers this form as proof that the nervous system plays an important rôle in the spasmodic element in the common exudative form of asthma as well, which fact sets asthma apart as a morbid entity distinct from bronchitis.

Comment.—Two important points must be considered in this case: First, the initial attack of asthma occurred within the last three weeks of life; and second, the presence of tumor masses pressing against the structures of the neck, mediastinum and lung. We question, therefore, whether this is to be considered as a typical case of bronchial asthma or whether it is rather to be classed as a symptomatic asthma due to a medullary carcinoma involving the respiratory tract.

Case 4. 1898. A. Fraenkel.[8] *History.*—Male, aged 63, carpenter. Previous illnesses: rheumatism and gout for years, bronchial catarrh since 60 and followed two years later by asthmatic attacks. About six weeks before death the patient entered the hospital suffering from gout, emphysema, chronic bronchitis and frequent asthmatic attacks. During attacks the lung borders were lowered and numerous râles were heard all over the chest. The sputum contained a few Charcot-Leyden's crystals but no Curschmann's spirals. The patient improved steadily for a time but died suddenly during a severe attack after a five-day period of freedom from attacks.

Necropsy Report: *Macroscopic Examination.*—Both sides of the heart are dilated. The lungs are distended and almost cover the pericardium. The right lung is slightly adherent to the chest wall. The bronchi on both sides are dilated, their mucosa is reddened and they contain a mucous material which can be pulled out threadlike. The bronchial glands are greatly enlarged and pigmented.

Microscopic Examination.—In the stained sections of the bronchial clots there are no typical spirals, but twisted threads, resembling fibrin, are present. The epithelium of the middle sized bronchi is detached from the basement membrane and forms twisted rows of cells which completely occlude some of the bronchi. Some of these cells are greatly elongated. The denuded wall appears thickened and contains many dilated capillaries and round cells. Some of the alveoli are emphysematous and some are compressed, while some contain a few blood cells. In some places the interstitial connective tissue is increased.

Comment.—To us, the most interesting points in this case are the history of a chronic bronchitis, the advanced age of the patient when the first attack occurred (62), attacks for only one year before death and the necropsy findings of an adhesive pleuritis and a thickening of the bronchial walls. It may be questioned whether this is a typical case of bronchial asthma or is a senile dyspnea due to a combination of a chronic bronchitis, emphysema and a failing myocardium.

Case 5. 1900. A. Fraenkel.[9] *History.*—Male, aged 48. For about one and a half years preceding death, the patient remained in the hospital suffering from almost daily severe asthmatic attacks, during which he raised a tenacious mucus containing Curshmann's spirals and Charcot-Leyden's crystals. The last severe attack resulted in death thirty-six hours later from collapse.

Necropsy Report: *Macroscopic Examination.*—The middle-sized and smaller bronchi of both lungs are almost or completely occluded by screw-shaped clots

8. Fraenkel, A.: Zur pathologischen Anatomie des Bronchialasthmas, Ztschr. f. klin. Med. **35**:559, 1898.

9. Fraenkel, A.: Zur Pathologie des Bronchialasthma, Deutsch. med. Wchnschr. **17**:269, 1900.

which can be pulled out with difficulty. In the larger tubes these clots are looser and several centimeters in length.

Microscopic Examination.—Stained (Bondi-Heidenhain) specimens of these clots show them to be true mucus, the threadlike central portions staining more intensely than the outer portions, which contain numerous eosinophil cells. In the smaller bronchi, from 0.15 to 0.03 mm. in diameter, isolated epithelial cells are elongated to about 20.4 microns in length, resting on a layer of small round cells; the ciliated borders of some are ruptured and the mucous content of the cell protrudes as a drop in the lumen, often fusing with a neighboring drop. In places these cells appear drawn out to form awl-like figures, in others they are heaped up to form layers two or three cells deep. The alveoli are almost free of secretion, containing only a few epithelial and eosinophil cells, and their walls contain dilated capillaries and collections of mononuclear and polynuclear cells. The walls of the small bronchi contain dilated capillaries and are infiltrated from the epithelium to the outer layer by cells, most of which are mononuclear and polymorphonuclear eosinophil cells. These cells are most numerous in bronchi containing glands and cartilages. Charcot-Leyden's crystals are found in places where the eosinophil cells are grouped.

Comment.—This seems to be a true case of bronchial asthma of several years' duration. The most important finding is the microscopic evidence of an extensive chronic infection of the bronchi. It is of especial interest that most of the cells infiltrating the bronchial walls are eosinophilic.

Case 6. 1905. Jezierski.[10] *History.*—Male, gardner, aged 63. Father died of asthma. Patient first had cough with dyspnea about five years before death and in the last two years of life the symptoms were so severe on two occasions that hospital care was needed. The symptoms consisted chiefly in attacks of severe expiratory dyspnea, sensation of fear, palpitation with tachycardia, pain in the epigastrium and cyanosis.

Physical examination showed a barrel-shaped chest, wide intercostal spaces, lowered lung borders, difficult expiration, and numerous dry and moist râles. After a six months' stay in the hospital the patient contracted a right lobar pneumonia and died two days later.

Necropsy Report: *Macroscopic Examination.*—The upper and middle lobes of the right lung are large and of liver-like consistency. The left lung is small and light and the mucosa of its dilated bronchi is reddened.

Microscopic Examination.—The bronchial lumina of the left lung, which is free from pneumonia, contain mucous masses in which are embedded cell remnants, well preserved ciliated epithelial cells often in rows, leukocytes, lymphocytes, erythrocytes and eosinophils. No fibrin is found. The well preserved epithelium is infiltrated by numerous round cells which invade all layers of the bronchial wall and in places are collected into groups resembling lymph glands. Beneath the epithelial layer the elastic tissue is markedly increased. There are also an unusual number of thin-walled closely-crowded capillaries around the tunica propria. The diaphragm shows some rarification and fatty infiltration. The neck muscles, the vagi and the cervical nerves show no changes, but the phrenic nerve contains groups of degenerating fibers. The right lung is a typical pneumonic lung.

Comment.—The clinical history of attacks of dyspnea for about five years before death in an old man and the microscopic evidence

10. Jezierski: Zur Pathologie des Asthma bronchiale, Deutsch. Arch. f klin. Med. **85**:342, 1905.

point to infection in the respiratory tract as a probable etiologic or complicating factor in the production of the asthmatic attacks, but the hereditary factor, the occupation and the presence of eosinophils point to an allergic pathogenesis. The most significant pathologic findings are the mucous masses in the bronchi, the cellular infiltration in the bronchial wall, the presence of eosinophil cells and the increase in elastic tissue.

Case 7. 1905. Jezierski.[10] *History.*—Female, aged 46, silk weaver. Asthmatic attacks began twelve years previously. Patient entered the hospital a short time before death suffering from dyspnea, cyanosis and tachycardia. The lung borders were lowered and there were many buzzing and whistling râles on expiration. Death followed collapse during a severe attack.

Necropsy Report: *Macroscopic Examination.*—Each pleural cavity contains about 200 c.c. of a bloody serous fluid. The left lung is markedly emphysematous and contains much blood. The bronchial lymph glands are anthracotic. Small wormlike mucous masses can be pressed from the small bronchi. The right lung contains fewer of these masses than the left.

Microscopic Examination.—The mucous masses contain many elongated ciliated epithelial cells, some of which are drawn out as long filaments, cuboidal cells from deeper layers of the epithelium, round cells, numerous eosinophil cells, some erythrocytes and cellular debris. These constituents vary considerably in numbers and distinctness in different sections. The epithelium of the bronchi is intact, except in a few places where it is penetrated by round cells which infiltrate the bronchial wall either diffusely or are collected in groups about vessels. This infiltration is so marked in the smaller bronchi that all structures are obscured. The elastic tissue is not increased and there are no new-formed blood vessels. The lung parenchyma is intact.

Comment.—The most significant pathologic findings are the occlusion of many bronchi by mucous masses and the extensive cellular infiltration of the bronchial walls. Many of these cells are eosinophils. While the microscopic evidence in this case points to infection as a probable etiologic factor in the production of the asthmatic attacks in this case, the clinical and necropsy evidence implicating the heart cannot be overlooked.

Case 8. 1908. A. G. Ellis.[11] *History.*—Coachman, aged 27, had been under treatment for tachycardia and bronchial asthma during the preceding year, and the day before death was admitted to the hospital during an asthmatic attack, with severe expiratory dyspnea, cough and cyanosis of face and extremities. The attack persisted and the patient collapsed and died the following day.

Necropsy Report: *Anatomic Diagnosis:* Hypertrophy of the left ventricle; dilatation of the right ventricle; pulmonary emphysema; purulent bronchitis; exudative bronchiolitis.

Macroscopic Examination.—The lungs are distended and entirely cover the pericardium. The right lung is slightly adherent to the thoracic wall and both lungs on sections show shiny, often greenish-colored plugs in the smaller bronchi.

Microscopic Examination.—Sections taken from various parts of both lungs reveal the following changes: The alveoli are distended only in patches and

11. Ellis, A. G.: Pathological Anatomy of Bronchial Asthma, Am. J. M. Sc. **136**:407, 1908.

those bordering the bronchi contain mucus, some leukocytes and a few eosinophil cells. The capillaries are uniformly engorged. The lumina of most of the smaller bronchi, from 0.13 to 0.16 mm. in diameter, are partly or entirely filled with a slightly granular mucus which is often arranged in distinct spiral form, the peripheral portions being less dense and containing a greater number of cells. These cells are chiefly polymorphonuclear leukocytes, among which are degenerated epithelial cells. The epithelium is mostly intact and averages about 7 microns in height. The bronchi, from 0.2 to 0.45 mm. in diameter, contain mucus, sometimes with spiral arrangement. Some of the epithelium is retained, other portions are separated en masse from the basement membrane and in one place an eroded blood vessel is exposed. In bronchi 0.8 mm. in diameter the amount of mucous material and desquamated epithelial cells varies, and some of the cells are much elongated (35 microns). In all these bronchi the mucus is arranged in layers, as is shown by the distinct longitudinal rows of cells. A few polymorphonuclear eosinophil cells are present in the lumina of all the bronchi. The walls of the bronchi and of the adjoining alveoli are infiltrated with leukocytes, the inner layer of the wall near the epithelium containing chiefly polymorphonuclear leukocytes, the outer layer containing chiefly large mononuclear cells with vesicular nuclei. Among these cells, especially in the middle sized bronchi, there are varying numbers of polymorphonuclear eosinophil cells. The capillaries in the walls are dilated and in some places small hemorrhages are found. The tunica propria and the basement membrane appear hyalin in some places. There is no change in the muscle or elastic tissue, and the connective tissue is not appreciably increased. The larger blood vessels of the lung contain more than the usual amount of blood and one contains a small thrombus.

Comment.—The most important findings in this case are pleural adhesions of one lung, occlusion of many of the smaller bronchi by mucous masses and the cellular infiltration of the bronchial walls. The extensive cellular infiltration of the bronchial wall shows that a chronic infection was present at death. Without considering the clinical data, these two facts point to infection as a possible etiological factor in this case of bronchial asthma, but the presence of many eosinophil cells suggests an allergic pathogenesis.

Case 9. 1909. Mönckeberg.[12] *History.*—Male, aged 29, mason. Previous illnesses, pneumonia in twelfth and again in eighteenth year. Psoriasis in twenty-fourth year. After serving in the army without any sickness from his twentieth to his twenty-second year, he first noticed in his twenty-fifth summer an irritating cough and dyspnea following a rapid march. He tried to be exempted from participation in some military maneuvers later that year because of this trouble, but failed. At this time, while in his quarters at night, he had severe attacks every four to eight days, and was later released from service. Following this, severe attacks came more frequently, often from five to six times daily, and for the rest of his life, about four years, he gradually grew worse, except for transient periods of improvement. A short time before death he entered the hospital because of dyspnea and a generalized edema. At this time he had a constant dyspnea which increased in severity, came in attacks every two or three hours and was relieved by coughing up much tough mucus, containing numerous spirals, eosinophil cells and leukocytes. The lung borders were not movable, and numerous vesicular and ringing râles were heard on expiration. The heart was rapid and irregular and the blood contained an increased number of

12. Mönckeberg, J. G.: Zur Pathologischen Anatomie des Bronchialasthmas, Verhandl. d. deutsch. pathol. Gesellsch. **14**:173, 1909.

eosinophile cells. The urine was not decreased in amount. Death followed a series of severe attacks.

Necropsy Report: *Anatomic Diagnosis:* Desquamative bronchial catarrh with hypersecretion of mucus, spiral formation, eosinophilia and asthmatic crystals; vesicular emphysema; bilateral complete adhesive pleuritis; isolated chronic and fresh tubercles in both lungs and in the hyperemic enlarged lymph glands.

Macroscopic Examination.—Well-developed and well-nourished male with generalized edema. Abdominal cavity contains much clear yellow fluid. Thorax barrel-shaped. Both pleural cavities are completely obliterated by adhesions. The right heart is markedly dilated and its walls thickened. The myocardium is a pale yellowish-brown color. No valvular changes. The mediastinal and bronchial lymph glands are large, soft and red. Both lungs are dilated. The cut surface of the lungs shows all the larger and most of the smaller bronchi to be completely occluded by tenacious, yellowish-white branching masses. The bronchial walls are thickened and show two distinct zones, a thin inner grayish-yellow zone and an outer reddish zone. The chief bronchi contain only some tenacious mucus not adherent to the wall. The remaining organs show the usual findings of a chronic passive hyperemia.

Microscopic Examination.—Most of the emphysematous end branches of the bronchi and the alveoli are empty, a few containing fluid and epithelial cells and having thickened walls, some of which are infiltrated by mononuclear and polymorphonuclear cells, chiefly eosinophils. The elastic fiber tissue is not increased, but the muscular tissue in the alveolar and lobular septa is increased. The pulmonary artery is thickened and sclerotic throughout and some branches contain organized thrombi. There are a few scattered new epitheliod miliary tubercles in the thickened cell-infiltrated connective tissue about the larger bronchi. The walls of the pulmonary veins are diffusely thickened. All capillaries are engorged by blood, and in places there are extravasations of erythrocytes into the tissue. The bronchioles up to the diameter of 0.2 mm. are either empty and with intact epithelium, or they contain bands of desquamated epithelial cells with interwoven cilia, a few "Herzfehlerzellen" and some mucus. There are no inflammatory changes in the thin muscular layer of these dilated bronchioles. The lumen of bronchioles, from 0.2 to 0.4 mm. in diameter, contains somewhat more well-preserved desquamated epithelium, round cells and detritus, and the walls contain a few mononuclear and polymorphonuclear eosinophil cells. The bronchial branches from 0.4 to 0.5 mm. diameter have a stronger muscular layer, which is frequently interrupted by diverticula or cryptlike protrusions of epithelium (Schleimhautausstülpungen). The epithelium is mostly intact, and at the crests of the folds is crowded together and appears elongated. The lumen contains cellular detritus, mucus, epithelial cells and round cells, often arranged in whorllike thickenings. The walls are infiltrated by many round cells. The walls of bronchi, from 0.5 to 1 mm. in diameter, contain small cartilages and mucous glands, the muscular layer is markedly developed and the cryptlike diverticula are more numerous and some are branched. The mucous glands which are in active secretion open into some of these diverticula. The broadened and hyalinized basement membrane continues into the diverticula, but the muscular layer is interrupted at the necklike opening of these structures. The epithelium is thickly crowded, and on the crests of the folds is arranged tuftlike and in places is much elongated, especially in the diverticula where these cells contain much mucus. The lumen of these bronchi is almost filled by mucus in which there are varying numbers of isolated or groups of desquamated ciliated epithelial cells, mononuclear and polymorphonuclear eosinophil cells, Charcot-Leyden's crystals and cellular detritus. These constituents are often arranged in twisted thickened lines and arranged in rows. The whole bronchial wall is thickened and infiltrated by round cells. In the bronchi, from 1 to 3 mm. in diameter, the walls are thickened, hyperemic and

thickly infiltrated by round cells, the lymphoid follicles and mucous glands are enlarged, the muscular layer hypertrophic, the diverticula more numerous, and the epithelium single-layered and greatly elongated. The content of the lumen is the same as that in somewhat smaller bronchi. In the chief bronchi mucus exudes from the gland ducts and spreads over the epithelial surface. The hyperemic bronchial glands contain a few fresh epitheloid tubercles.

Comment.—The most important points in this case are: History of pneumonia at 12 and again at 18; relief from dyspnea after raising masses of mucus, bilateral adhesive pleuritis, complete occlusion of most of middle sized bronchi by mucus, thickening of the bronchial walls, apparent hypertrophy of bronchial musculature, cryptlike structures in the bronchial walls, cellular infiltration, largely eosinophilic, of the bronchial walls, enlargement of the mucous glands and epitheloid tubercles. The history of pneumonia, the bilateral adhesive pleuritis and the cellular infiltration of the bronchial walls all point toward infection as a possible etiology in this case, but an allergic pathogenesis is also indicated by the extensive eosinophilia. The hypertrophy of the musculature is no doubt due to the four years of almost continuous asthmatic attacks of dyspnea. The finding of large mucous glands and of the cryptlike structures in the bronchial walls is significant.

Case 10. 1911. Hermann Heizer.[13] *History.*—Child, aged 2 years. Family history negative. Eczema since fourth month. First attack of dyspnea at 9 months. Attacks often followed exposure to wind or dust, and were accompanied by cyanosis, chest noises and the production of a large amount of tenacious sputum. Death followed an attack.

Necropsy Report: *Macroscopic Examination.*—Lungs distended. Heart enlarged and right ventricle thickened. Trachea and large bronchi contain a purulent secretion. All lymph glands of the body are greatly enlarged.

Microscopic Examination.—The alveoli vary in size, some of their walls are thinned and some are ruptured. Some bronchi are dilated and occluded by masses of mucus containing leukocytes and epithelial cells. The walls of the large bronchi are thickened by infiltrating cells and distended capillaries. The bronchial mucous glands are markedly enlarged and their ducts filled with mucus.

Comment.—The most significant findings reported are the history of eczema for months and of attacks following exposure to wind and dust, the generalized enlargement of the lymphoid tissues, hypertrophy of right ventricle, thickening of the walls of the bronchi by cellular infiltration and capillary distension and the occlusion of many bronchi by mucus. The clinical history points to both foods and inhaled dust (animal or plant) as probable etiologic factors in the production of the attacks of dyspnea.

Case 11. 1913. M. M. Tichmeneff.[14] *History.*—Female, aged 29, college student. No hereditary peculiarities. Nasal catarrh since early childhood. Following an attack of croup in the eighth year, asthmatic attacks lasting from

13. Heizer, H.: Dissertation, München, 1911.
14. Tichmeneff: Bronchial Asthma with Death, Prakt. Vratch **12**:562, 1913.

two to three days came on four or five times each year, usually in the spring and fall, and on two occasions in later years required hospital care. During an attack three weeks before death she entered the hospital with a severe very productive cough, and on examination showed the characteristic posture, difficult whistling expiration, limited excursion, some emphysema, numerous whistling, buzzing râles, normal heart and normal digestive, nervous and urinary systems. Pulse, 112; temperature, 37.5 C. Hemoglobin, 87 per cent.; erythrocytes, 5,690,000; leukocytes, 15,200, showing by differential count: lymphocytes, 26 per cent.; transitionals, 4 per cent.; neutrophils, 60 per cent, and eosinophils, 7 per cent. Sputum contained many pus cells, Charcot-Leyden's crystals, Curshmann's spirals and eosinophil cells. Urine contained a trace of albumin. The attack of asthma subsided in three to four days and pneumonic symptoms appeared. Two days before death a severe gastro-intestinal hemorrhage began and continued until death.

Clinical Diagnosis.—Bronchial asthma; left-sided exudative pleuritis and pneumonia; ulcer of the stomach or duodenum.

Necropsy Report: *Macroscopic Examination.*—The left pleural cavity is partially obliterated by fibrous adhesions and contains 300 c.c. of a purulent exudate. The left upper lobe contains a small cavity and a row of small consolidations from which pus exudes on pressure. The right lung is soft and exudes a mucopurulent fluid. The pericardial sac contains a small amount of clear yellowish fluid. Heart: 9 by 9 cm.; myocardium pale; large vessels unchanged. Spleen pale and flabby. Liver yellowish. Kidneys pale. Stomach mucosa normal. Large duodenal ulcer 5 cm. from the pylorus showing eroded blood vessels.

Microscopic Examination.—Slight brown atrophy of the heart. In the lumen of some of the small bronchi there is an exudate which contains many round cells and leukocytes. The tightly crowded columnar epithelial cells are much elongated and are arranged in several rows as if in increased proliferation. All the layers of the bronchial wall are considerably thickened, due to an increase in connective tissue, round cell infiltration, and hypertrophy of the muscular layer. Some of the bronchi are dilated, their walls markedly infiltrated by round cells, and are surrounded by areas of pneumonic infiltration. The cavity is a typical bronchiectatic cavity.

Comment.—The most significant pathologic findings are blood eosinophilia, 7 per cent.; adhesive pleuritis and increase in thickness of the bronchial walls due to accumulation of round cells, hypertrophy of muscle layer and increase in connective tissue. This appears to be a true case of bronchial asthma in which death was due to an intestinal hemorrhage. The etiology of the attacks cannot be determined, for the history and the clinical and necropsy evidence point to infection as a possible causal agent, while the eosinophilia points to an allergic pathogenesis.

Case 12. 1916. Marchand.[15] *History.*—Female, aged 53. Hereditary peculiarities: Mother died of asthma and capillary bronchitis. History of present illness: Capillary bronchitis since 17 and asthmatic attacks since 30. She first entered the hospital in her forty-third year suffering from bronchial asthma, pulmonary emphysema and chronic laryngitis, and left six weeks later much improved. Ten years later, about two weeks before death, she returned to the hospital, following an attack of six weeks' duration, suffering from dyspnea, severe cough with much expectoration, headache, insomnia and

15. Marchand, F.: Beitrag zur Pathologie und pathologischen Anatomie des Bronchialasthmas, Beitr. z. path. Anat. u. z. allg. Path. **61**:251, 1916.

loss of appetite. The attacks came chiefly at night. The examination showed a marked dermographia; cyanosis of the lips and fingers; barrel-shaped chest; labored respiration; lung borders on both sides anteriorly at the seventh rib and posteriorly at the twelfth spinous process; boxy percussion note all over the lungs; deep humming inspiration; prolonged loud, shrill, whistling expiration; heart action accelerated but with tones clear. Roentgen-ray examination: Diaphragm contracted and with limited motion; distinct hilum shadows; marked pulsation of the right ventricle.

She raised a large quantity of a tenacious mucous sputum which contained some spirals, numerous eosinophil cells, but no crystals. The blood showed a leukocyte count of 12,600, of which 12 per cent. were eosinophil cells. Temperature, 37 C. The blood serum was very toxic for animals, 0.05 c.c. causing death of a guinea-pig. After standing one and one-half hours, the serum contained many crystals. Following a severe attack of two hours' duration, the patient collapsed and died.

Necropsy Report: *Anatomic Diagnosis.*—Severe mucous bronchitis. Vesicular emphysema. Black circumscribed subpleural indurations in both apices. Recent tuberculosis and hypertrophy of the bronchial lymph glands. Fibrous induration and calcification of bronchial glands. Nodular colloidal goiter. Right ventricular hypertrophy and early fatty degeneration of the myocardium. Hyperemia of brain. Chronic perimetritis. Slight atrophic granular kidney. Hypertrophy and hyperemia of spleen.

Macroscopic Examination.—The body is that of a small well-preserved female, weighing 48 kg. The tips of the fingers are cyanotic. The thorax is not arched. The brain shows no signs of disease. The diaphragm is at the sixth rib on the right side and in the fifth intercostal space on the left side, and is contracted. The lungs do not collapse when the chest is opened, almost completely cover the pericardial sac, and are attached to the chest wall by many stringlike adhesions. A fatty remnant of the thymus is present. The pericardial sac contains a small amount of a clear yellowish fluid. The heart is contracted and the right ventricle appears to be increased in size. The valves are slightly thickened. The foramen ovale is not completely closed. The alveoli in the anterior borders of the lungs are markedly dilated. The cross section of the upper lobes are bluish gray and the outer portions are soft and edematous. The lower lobes are firmer and filled with blood. Most of the smaller bronchi in both upper and lower lobes are completely filled by a tenacious transparent material, which on cross section protrudes and can be drawn out as threads several centimeters long. In some places these threads appear as yellowish, opaque masses. The larger bronchi contain a more fluid yellowish gray material. The lower lobes are fairly dry except for the congested areas. Beneath the pleura of both apices there are a few hard black thickenings 1 cm. in diameter. The mucosa of the trachea, larynx and nasal cavities is not essentially changed.

The thyroid is slightly enlarged and contains a small colloid nodule.

The bronchial glands are large and soft, the gland at the bifurcation of the trachea being as large as a pigeon egg and containing numerous grayish nodules. One gland at the hilum of the right lung contains a calcified nodule.

The spleen is enlarged, firm and congested.

Microscopic Examination.—Blocks of tissue from all parts of the lungs, from bronchial glands, trachea, ethmoid region and the middle turbinate were fixed in liquor formaldehyd, alcohol or Zenker's fluid, and the sections stained with various stains.[16]

The upper lobes show a vesicular emphysema with the formation of some large vesicles. The bronchioli respiratorii are for the most part empty, vary considerably in size up to 0.1 mm. and are lined by a low epithelium, some of

16. The detailed descriptions of the sections and of the special form of crystals are omitted in this paper.

which is ciliated and measures 0.009 mm. Bronchioli, from 0.5 to 1 mm. in diameter, are filled by an homogenous mass containing a few cell elements which is adherent to the epithelium. In some of the smaller bronchioli a transparent, almost homogenous, layer covers the epithelium, the central portion being patent. This layer stains poorly, contains a few mononuclear cells, alveolar epithelium, and shows on high magnification a netlike structure which stains with Weigert's stain like fibrin. The cylindrical epithelium of the smaller, middle-sized bronchioli is mostly intact, and is heaped up and deformed only in the angles between folds. There are no mucous glands present, but the muchematein stain shows numerous goblet cells in bronchioles 1 mm. in diameter. The smaller bronchi contain a longitudinally streaked mucous material, with rows of cells and spirals having distinct central threads. The cellular content of the smaller bronchioles is scanty, while in the larger there are numerous round or elongated polymorphonuclear eosinophil cells which are often arranged in clumps, numerous polymorphonuclear leukocytes, some mononuclear, large, pale, swollen cells (alveolar epithelium), and in the larger bronchioli a few mast cells. In bronchioli, from 0.5 to 0.8 mm. in diameter, the epithelium varies in thickness from 0.025 to 0.04 mm., and in bronchi from 3.5 to 4 mm. in diameter, it varies from 0.05 to 0.06 mm. in thickness. The content of the larger bronchi and the trachea is composed of much well-preserved ciliated epithelium, many large markedly swollen round cells which contain droplets of myelin material, granular cells resembling eosinophil cells, a few Charcot-Leyden's crystals and numerous crystals which are different from the Charcot-Leyden's crystals. The trachea also contains several clumps of bacteria, most of which resemble the influenza bacillus. The epithelium of the larger bronchi and the trachea contains numerous goblet cells and is infiltrated by a few leukocytes, eosinophil cells and mast cells. The nasal mucosa over the free borders of the turbinates is thickened and infiltrated by cells.

The walls of all the bronchioles are more or less infiltrated by round cells of different kinds, the lymphoid elements being most numerous and often grouped to from small nodules under the epithelium and in the outer layer. The other kinds of cells, which frequently separate the muscle bundles, are: (*a*) large mononuclear basophilic cells often grouped around the acini of mucous glands; (*b*) eosinophils; (*c*) mast cells varying greatly in size and lying chiefly close to the epithelium; (*d*) a few polymorphonuclear leukocytes.

The mucous glands of the larger bronchi and the trachea show a more or less active secretion with transformation of the cylindrical cells of the duct into goblet cells. The gland cells are for the greater part markedly swollen and filled with mucus.

The blood smears and rib-marrow preparations contained numerous eosinophil cells, while the spleen contained only a few such cells. The lymph glands at the bifurcation of the trachea are enlarged and contain a small tubercle with some caseation and giant cells.

Comment.—The most important pathologic findings are: complete occlusion of most of the smaller bronchi, cellular infiltration in the bronchial wall, enlargement of the bronchial mucous glands and the numerous eosinophil cells in the bronchial wall. The history of bronchitis since the seventeenth year and of asthma beginning in the thirtieth year, the necropsy finding of marked cellular infiltration of the bronchial walls, point to infection as a probable etiologic factor in this case, but the eosinophilia indicates an allergic pathogenesis.

Case 13. 1916. Marchand.[35] *History.*—Mrs. T. B., aged 45. Previous history: The patient has had two severe attacks of asthma in the year preceding her entrance into the hospital where she had a third fatal attack.

NECROPSY REPORT: *Anatomic Diagnosis.*—Marked fibrinous bronchitis with obturation of the left bronchus; fibrinomucous tracheitis; purulent bronchiolitis; marked pulmonary emphysema; healed right apical tuberculosis; fibrinous lobular pneumonia; cyanosis of the liver, spleen and kidney; small hemangioma of the liver; accessory spleen in tail of pancreas.

Macroscopic Examination.—Small emaciated woman with scanty fatty and muscular tissues. Diaphragm on right side at fourth rib and on left at fourth interspace. Heart not enlarged. Left ventricle, 8 mm. and the right 3 mm. in thickness.

The lungs do not collapse when the chest is opened. There are a few adhesions to the sternum. Both lungs are large and light. The posterior portion of the right upper lobe contains a few hazelnut-sized areas with caseous centers. On cut section the lung tissue is dry and contains little blood. The left chief bronchus and its branches are almost completely occluded by a tenacious exudate. The middle-sized bronchi contain glassy tenacious mucus and the finer branches contain a more fluid yellowish turbid exudate. The mucosa of the chief and smaller bronchi is swollen and reddish. The trachea contains an adherent mucous mass which extends into the right main bronchus. The peritracheal and peribronchial lymph glands are enlarged and contain no nodules. The liver extends two fingers below the costal margin. The spleen is not enlarged.

Microscopic Examination.—The lungs were fixed in liquor formaldehyd and preserved in alcohol. A large number of blocks of completely infiltrated and air-filled portions of the tissue were taken, but the original positions of these blocks could not be established later. The cross section of the exudate filled bronchi appears as whitish round disks in both the infiltrated and air-containing portions. The parenchyma of the infiltrated portions of lung resembles a catarrhal lobular pneumonia and contains many polymorphonuclear neutrophil cells, but no eosinophil cells. The parenchyma of the air-containing portions shows a moderate degree of emphysema.

The content of the lumen of the small and middle-sized bronchi in both air-containing and infiltrated portions is made up of homogenous, transparent mucus which has in many places a streaked appearance and which contains many closely crowded polymorphonuclear neutrophil cells but no eosinophils. The cylindrical epithelium is fairly well retained and contains a few globlet cells. The walls of the bronchi are thickened and contain, especially in the outer layer and between muscle bundles, many mononuclear lymphoid cells, although the blood-filled vessels contain no cells of this type and show no evidence of their migration. In some of these bronchi there are numerous eosinophil cells, either rowlike, scattered or clumped between connective tissue bundles, and most numerous in the inner layers. The eosinophil cells also intermingle with the lymphoid cells in the outer portion of the lymph nodules. There is no definite relation between accumulations of eosinophil cells and the blood vessels. These cells vary in shape and size, in number, distribution, intensity of staining property and size of granules, and in morphology and size of the nucleus. All transitions of nuclei from the mononuclear to the polymorphonuclear are visible. There are no Charcot-Leyden's crystals among the eosinophil cells or in the bronchial wall.

The lumina of some of the larger bronchi, as shown in pictures, are completely occluded by a transparent exudate which is adherent to the wall and is continuous with the content of the gland ducts. The epithelium is partially desquamated and in places necrotic. The tissue of the walls is markedly infiltrated by cells, chiefly polymorphonuclear neutrophils and eosinophils. The latter predominate in some portions of the wall, and with the neutrophils are seen penetrating the hyalin tunica propria. A few Charcot-Leyden's crystals also are present. The blood vessels are engorged with erythrocytes, among which there are a few mononuclear and polymorphonuclear leukocytes, none being eosinophilic. The cells of numerous large mucous glands in the wall are

distended by mucus, as are also many of the duct cells. In some portions of the wall there are circumscribed or diffuse collections of lymphocytic cells.

The left chief bronchus from 1 to 3 cm. from the bifurcation is almost occluded by an exudate and its epithelium partially desquamated. The mucous gland ducts are filled by mucus and the cells of the ducts and acini are distended. The mucosa is markedly infiltrated by round cells, partly mononuclear and partly polymorphonuclear, and many eosinophil cells. Some Charcot-Leyden's crystals are present in the walls and on the surface of the mucosa. The content of the lumen is composed of mucus and fibrin which show a spiral arrangement. In some places well-formed spirals with central threads are seen.

Many eosinophil cells and Charcot-Leyden's crystals are found in the mucosa of the trachea. In some of the thin paraffin sections small groups of organisms resembling the diphtheria bacillus are seen.

Comment.—The most significant findings in this case are: (1) The complete occlusion of many of the larger, middle sized and smaller bronchi by mucous masses. (2) Marked cellular infiltration of the walls of the bronchi. (3) Presence of eosinophil cells, scattered or grouped, in the walls of the trachea and some of the bronchi. (4) Presence of Charcot-Leyden's crystals in the walls of the trachea and in some of the bronchi. (5) Distension of the cells of the mucous glands and their ducts by mucus.

The etiology of the asthmatic attacks in this case has not been determined. The history of the asthmatic attacks coming on in the last year of life in a person 45 years of age, and the evidence of infection in the walls of the bronchi, point to bacteria as probable factors in the production of the attacks, but the extensive eosinophilic infiltration of the bronchi points again to an allergic pathogenesis.

Case 14. 1918. Marchand.[17] *History.*—Male, aged 48, worker in chemical factory. Family history: Aunt had asthma. Previous illnesses: None. When about 44 years of age he began work in a chemical factory and frequently inhaled poisonous fumes. Before quitting this work, two years later, he had his first attack of asthma after exposure to fumes. After this he often had marked dyspnea on exertion and his work was interrupted frequently, the dyspnea being always more noticeable in damp weather. After a free period during the following summer the attacks returned, accompanied by cough with the raising of a tenacious mucus, and the patient was often compelled to remain in bed for several days. About ten weeks before death both legs began to swell and he entered the hospital with the following findings: Edema and cyanosis of face, high grade inspiratory dyspnea, prolonged whistling expiration; lung lowered and immovable; heart and abdomen negative; sputum scanty, tenacious and containing crystals and eosinophils; urine negative. The blood was examined several times during the stay in the hospital and showed an eosinophilia averaging about 4 per cent. The blood pressure ranged from 135 systolic and 85 diastolic on entrance to 86 systolic and 60 diastolic two weeks before death. The patient's condition gradually became worse and about nine weeks after entrance, he died.

Necropsy Report: *Anatomic Diagnosis.*—Catarrh of the bronchial mucosa; catarrhal rhinitis with retained mucus in the left maxillary sinus; diffuse

17. Marchand, F.: Ein neuer Fall von Asthma Bronchiale mit anatomischer Untersuchung, Deutsch. Arch. f. klin. Med. **127**:184, 1918.

vesicular pulmonary emphysema; slight hypertrophy of the right ventricle; bronchial asthma.

Macroscopic Examination.—The lower part of the thorax is widened. The lungs are expanded and cover the heart. The bronchi up to the smaller branches are filled by yellowish-white tough masses which can be pulled out like threads. The left antrum of Highmore is completely filled by a tough opaque bluish secretion. The bronchial glands are not enlarged. The right auricle and ventricle are dilated and the walls thickened.

Microscopic Examination.—The mucous membrane of the nasal septum, turbinates and trachea contains many distended capillaries and is infiltrated by round cells, many of which are eosinophilic. The fresh secretions from the trachea and principal bronchi contain many prismatic crystals similar to those described in a previous case. (Case 12. Marchand's first case.) The lumen of the bronchi 5 mm. in diameter contains only a small amount of mucus in which are desquamated cells, leukocytes, round cells and a few eosinophils. The epithelium is mostly desquamated. The mucosa is unevenly thickened, its capillaries engorged and all layers contain numerous eosinophil cells which are often arranged in masses near the hypertrophied muscle layer. The mucous gland cells are swollen and contain no eosinophil cells. Smaller bronchi have a similar structure and are filled with mucous masses which have typical spiral arrangement. The smallest bronchioles and the alveoli are not essentially changed. One lymph gland contains a small tubercle.

Comment.—The most important points in this case are the complete occlusion of many smaller bronchi, enlargement of the mucous glands, hypertrophy of the muscle layer and the large number of eosinophilic cells infiltrating the bronchial walls.

Case 15. 1921. N. Kamchorn and A. G. Ellis.[18] Male. Siamese, actor and fishmonger, aged 52. Family history of asthma in four generations: Father's mother; father; one uncle, one aunt, and one child. Infrequent asthmatic attacks began in childhood and gradually became more frequent. Three months before admission to the hospital the feet and legs began to swell. On entrance eleven days before death, there was a marked edema, cyanosis and respiratory dyspnea. Coughing produced a tough white sputum. The heart sounds were faint and râles were heard over the bases of both lungs. Treatment gave no relief and death resulted from failure of the heart.

Necropsy Report: *Anatomic Diagnosis.*—Summary: Hypertrophy and dilatation of right ventricle; dilatation of right auricle and left ventricle; bilateral chronic adhesive pleuritis; partial atelectasis of left lung and lower lobe of right lung; vesicular and interstitial emphysema of right upper and middle lobes; acute bronchitis of left lung and right lower lobe; congestion of spleen, kidneys, stomach and liver; anasarca; multiple serous effusions.

Macroscopic Examination.—Body edematous. All serous cavities, except the obliterated left pleural sac contain much fluid. The right ventricular wall is 1 cm. thick. The left lung and the right lower lobe are collapsed and their cut surfaces are dark bluish red in color. The middle sized and smaller bronchi contain a moderate amount of tenacious mucus and the mucosa is intensely red. The right upper and middle lobes are overdistended but on pressure and on incision exude frothy serum. The bronchi in these lobes are empty and the mucosa is pale in color.

Microscopic Examination.—The bronchial exudate contains much mucus, typical Curshmann's spirals, columnar epithelial cells and an occasional eosinophil cell. The epithelium of the bronchioles in the collapsed portion of the lungs is desquamated and its place occupied by polynuclear and mononuclear leuko-

18. Kamchorn, N., and Ellis, A. G.: Am. J. M. Sc. **161**:525, 1921.

cytes. The bronchial walls are infiltrated by mononuclear cells. The epithelium of the middle sized bronchi is partially desquamated. There is no evidence of increased fibrous tissue and of thickening of the bronchial wall and the muscularis mucosa does not appear abnormal. The bronchi in the distended portions of the lung have much less marked changes.

Comment.—The following points are the most important in this case: The history of asthmatic attacks since childhood and the microscopic evidence of an extensive respiratory infection sometime during life, as shown by the extensive round cell infiltration of the subepithelial layer of the bronchial tree. The hypertrophy of the wall of the right ventricle is probably due to the extra load thrown on pulmonic circulation during attacks. The remaining pathology in this case is probably dependent on the failing heart.

MODE OF PROCEDURE EMPLOYED IN THIS STUDY AND A DESCRIPTION OF THE NORMAL BRONCHIAL STRUCTURES

Before discussing in detail the various changes involving some of the different structures which may be implicated in the production of bronchial stenosis, i. e., the smooth muscle fiber system, the mucosa, the glandular system, the blood vessels and lymphatics and the nerves, we deem it proper to give a brief description of the finer anatomy of the normal bronchial structures.[19]

When possible, in all cases studied, the intact lung was fixed either in liquor formaldehyd or in Zenker's fixing fluid, and each lobe was sectioned as described so that the blocks of tissue gave either cross or longitudinal views of the main branching of the bronchial tree from the hilum to the pleural surface (Fig. 1). The cross sections for microscopic study were taken as nearly as possible from the plane at right angles to the central axis, as it is clearly seen that sections through oblique planes must give a distorted picture of the structures. The sections were stained with hemotoxylin and eosin and Van Gieson's and Mallory's connective tissue stains.

We have examined microscopically the lungs of several persons which should have shown, according to the history and the macroscopic examination, practically normal structures, yet we have found in all of them some pathologic changes, i. e., foci of round cell infiltration, tubercles, calcified cartilages, capillary dilatation or other changes

19. Oppel: Lehrbuch der vergleichenden mikroskopischen Anatomie **6-7.** Miller, W. S.: Reference Handbook Medical Sciences; Am. Rev. Tuberc. **2:** (May) 1918; **2:** (Jan.) 1919; Anat. Rec. **5:** (March) 1911; Bull. Robert Koch Soc. for Study of Tuberc. (Feb. 11) 1913; (April 20) 1916; Anat. Anz. **28:** 1906; Contributions to Embryology, No. 38. Larsell, O.: Nerve Terminations in the Lung of the Rabbit, J. Compar. Neurol. **33:**105, 1921. Baltisberger, W.: Ueber die glatte Muskulatur der Menschlichen Lunge, Ztschr. f. Anat. in Entwicklungsgeschichte **61:**249, 1921.

which distorted the normal picture in some particular. These findings, in accordance with similar reports from others, show that few of us escape the marks left by acute or chronic respiratory infections which are capable of altering the normal tissue.

The principal bronchi enter the hilum of the lung and divide and subdivide quickly several times into smaller tubes, which when they are reduced in size to from 0.5 mm. to 1 mm. inside diameter, are termed bronchioles. These bronchioles divide to form the atrium, air sac (infundibulum), and the air cell (alveolus).

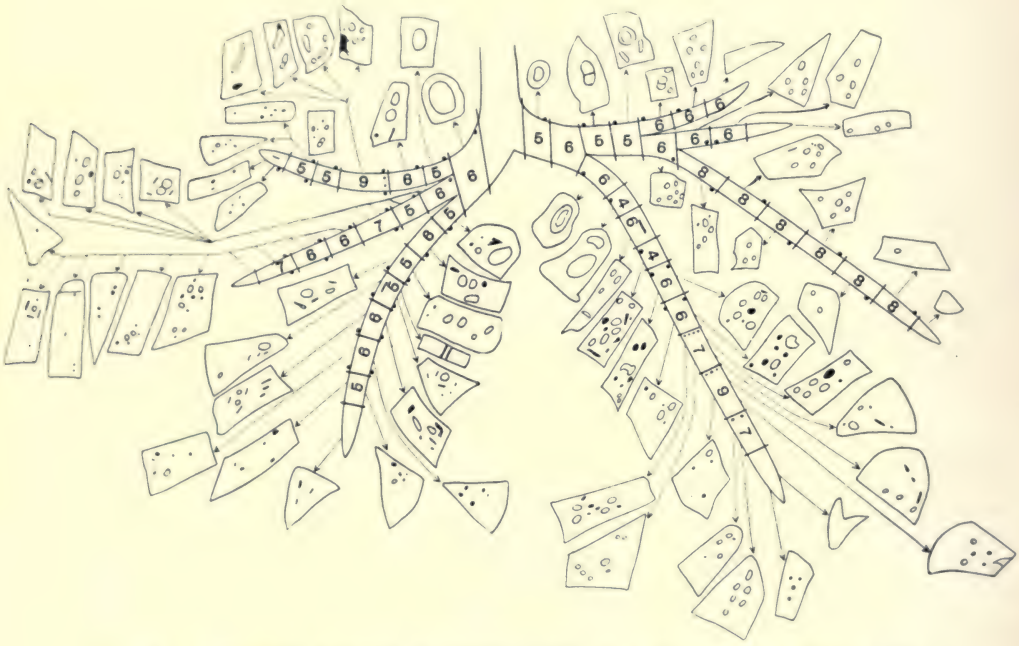

Fig. 1.—Diagram showing the method used for obtaining the blocks of lung tissue which contain the parts of the bronchial tree studied. The figures give the actual thickness in millimeters of the blocks used. The black dots along the bronchial margin indicate the side of the block from which the sections were taken for study. The measurements and sections shown in this diagram correspond to those actually obtained in Case 6.

The structure of all these divisions is, in general, the same, the arrangement, diminution or disappearance of any layer being incidental to the reduction in the size of the tube. We shall discuss briefly the following structures: The epithelium, basement membrane, subepithelial tissue, muscle, mucous glands and the outer fibrocartilaginous layer.

Epithelium.—The epithelium in the trachea and larger bronchi is made up of two or three layers, a deep layer of small, closely approxi-

mated cells, which in places has the appearance of two layers of cells, and a lining layer of ciliated columnar cells from which processes extend and attach to the basement membrane. In this lining layer there are also the goblet cells, which are most numerous in the larger bronchi and gradually disappear as the size of the tube decreases, but may be found even in the bronchioles. The epithelium also gradually changes in character from the layered ciliated type in the large bronchi to the single layer of ciliated or nonciliated cuboidal cells or flat cells in the bronchiole. This epithelium throughout is thrown into shallow longitudinal folds.

Basement Membrane.—The well-marked basement membrane on which the epithelium rests forms a thin compact layer, containing a few spindle shaped cells, and is pierced here and there by gland ducts and by processes passing into the epithelium.

Subepithelial Tissue.—The layer of loose connective tissue which is found just beneath the epithelium in all the divisions of the bronchi down to the bronchiole, has been designated by us the subepithelial connective tissue layer. It contains numerous capillaries, fine elastic fibers, small round cells and connective tissue cells and intermingles in its outer portion with the coarser elastic fiber layer. This latter layer is prominent in all the divisions of the bronchi down to and including the bronchioles, forming distinct longitudinal bundles of fibers which show some irregularities in the direction where gland ducts, vessels, etc., penetrate it. In these places the fibers may appear to form distinct circular bundles.

Muscle.—The description of the nonstriated muscular system of the bronchial tree and the lung parenchyma in histologies, almost without exception, is very meagre and misleading, and few observers, who have made no special study of this system, appreciate its complicated structure, extent and importance. In the trachea and in the main bronchi with similar structure, the muscle tissue is practically limited to the posterior membranous part where it forms bundles that extend between the ends of the incomplete ring cartilages and the interval between them, but, when these cartilages are replaced by irregular plates in the further divisions of the bronchi, the muscle forms a more or less continuous layer, the so-called "ring muscle" layer. The fact is, that, in man at least, this layer is not a true ring muscle, except probably in the finer bronchi, but is made up of short bundles which join each other in such a manner that a netlike sheath encircling the tube is formed, the general direction of the fibers being circular. As the tube diminishes in size, the muscular layer becomes thinner and thinner, and in the respiratory bronchiole and in the lung parenchyma it is represented by only a few delicate fibers. In certain lower animals, e. g., the turtle,

the distribution of smooth muscle fibers in the lung parenchyma is very striking.

The layer which connects the muscularis to the fibrocartilaginous layer consists of areolar tissue and contains mucous glands and mucoserous glands, blood vessels, nerves, and adipose tissue. This layer is best developed in the larger bronchi and, as the size of the tube diminishes, becomes progressively thinner, the glands being rarely found in tubes less than 1 mm. inside diameter.

Glands.—The gland system, like the muscle system, has not received the attention of the histologist that it deserves. The glands are of the tubulo-alveolar variety and are distributed chiefly between the cartilages and the muscle layer about the whole circumference of the bronchus, but in middle sized and smaller bronchi they are best developed in the tissue between the edges of the cartilage plaques and may even appear outside the cartilages. They are chiefly mucous glands but contain also a few crescents of Gianuzzi. The acini are lined by columnar epithelium and the ducts which often form ampullae-like widenings on entering the mucosa are lined by cuboidal ciliated epithelial cells. In certain animals, especially in rodents, gland tissue is absent in all the bronchial divisions within the lung. In others where glands are present the proportion of mucous to serous gland tissue varies greatly, thus in the sheep and the dog the serous glands are in preponderance while in cattle the amounts of serous and of mucous gland tissue are about equal.

Fibrocartilage Layer.—The outer layer of the bronchial tree is composed chiefly of connective tissue which in all except the finer divisions of the bronchi contains cartilages. These cartilages in the trachea and the primary bronchi form incomplete rings extending over the ventral and lateral walls, but in further divisions of the tube are broken up into irregularly shaped plates of various sizes and are disposed over the whole circumference of the tube. These plates gradually diminish in size and number and are rarely present in tubes with an inside diameter of less than 1 mm. The connective tissue, however, continues as a distinct layer into the much smaller divisions. It is interesting to note here that cartilages are absent in that portion of the bronchial tree within the lungs of some small animals, chiefly rodents.

Lymphoid Tissue.—This is found in all the main branchings of the bronchial tree, the amount varying according to the size of the tube. There are a few scattered cells of the lymphoid type in the subepithelial layer but true follicles and nodes are found only outside the muscular layer. Here these structures may lie between the muscle and cartilages, in the tissue between the cartilages, or as is usual for distinct nodes, outside the cartilages. The distinct nodes are usually located at the

point of bifurcation of the tube. Small lymphoid follicles are also found scattered in the wall of small bronchioles and even among the alveoli or beneath the pleura. Distinct glands are found about the trachea and larger division of the bronchi.

Blood Supply.—The blood supply of the bronchial tree is furnished by a special set of vessels, the bronchial arteries, branches of which are found in all the divisions of the bronchus down to the bronchiole. The main branches of this artery are situated in the outer fibrous coat of the bronchus, numerous radicles being given off to form plexuses which supply blood to the glands, muscles and other structures, the capillary network being especially rich in the subepithelial layer.

Nerves.—Nerves and nerve ganglia are found in the walls of all divisions of the bronchial tree down to the bronchiole, usually lying in close apposition to the branches of the bronchial artery.

REPORT OF CASES

CASE 1.—Mrs. B., aged 55, married, American, was first seen by one of us (K.) in April, 1915, suffering from frequent severe attacks of spasmodic dyspnea.

Family History.—Negative.

Previous History.—At 44 she had had a severe tonsillitis and one attack of severe abdominal pain (appendicitis?), but after that time she was well until April, 1913, when she suffered from an acute coryza and severe cough lasting three weeks. During the following summer (1913) she was very nervous and irritable, and in August she went to the White Mountains. Then she first noticed wheezing sounds in the chest at night. During the latter part of August and in September she had frequent head colds accompanied by severe coughing attacks, and in the latter part of September, following a severe prolonged coughing spell, the first distinct attack of spasmodic dyspnea occurred. These attacks of coughing and dyspnea increased rapidly in number and severity, and by October 10 they were coming four and five times daily, but were usually almost instantly relieved by small subcutaneous doses of epinephrin (1:1000). While visiting in the South in November and December she had complete relief from dyspneic attacks but the severe cough persisted, although usually relieved quickly by inhaling the smoke of stramonium leaves. While returning North in January, 1914, she contracted a severe cold and the dyspneic attacks returned with increased severity, forcing her to stop off at Atlanta, Ga., where she remained under a physician's care for about three months, apparently suffering from a bronchopneumonia. In April, in Chicago, the paroxysmal attacks of dyspnea again returned with increased frequency and severity and were incompletely relieved by subcutaneous injections of epinephrin, but the troublesome cough was relieved by small doses of potassium iodid. The attacks of dyspnea and coughing persisted to a greater or lesser degree all summer while traveling in the West. In September, 1914, she entered a Chicago hospital for observation by a local internist. There a tooth infection was cleaned up and a vaccine, made from her sputum cultures, was administered, but these measures seemed to give only a transient relief of symptoms. Following a pneumococcus infection in February, 1915, with a rise in temperature, she had almost complete relief from both the dyspneic attacks and the harassing cough until the latter part of March, when both returned in a severe form. At this time she entered the Presbyterian Hospital in Chicago.

Laboratory Examination.—Temperature, 98.6 F.; pulse, 96; respiration, 24. Blood pressure, 115/75.

Blood: Hemoglobin, 95 per cent.; leukocytes, 14,000; red cells, 5,700,000. Differential count: Small lymphocytes, 10 per cent.; large lymphocytes, 10 per cent.; large mononuclears, 5 per cent.; transitionals, 2 per cent.; neutrophils, 62 per cent.; eosinophils, 11 per cent.

Urine: Acid; specific gravity, 1.020; no albumin, sugar, acetone, or casts; few leukocytes; no blood; indican, a trace.

Sputum: Very abundantly produced, thick, mucoid, stringy with white particles; no Charcot-Leyden crystals, Curshmann's spirals or blood, a few eosinophils, but no polymorphonuclear neutrophil leukocytes.

Feces: Negative for blood and pus.

Physical Examination.—Skin, pale and dry, with cyanotic hue of the lips, nose, fingers and nails. No palpable glands. Throat, nose and sinuses negative (Dr. Shambaugh). Teeth negative (Dr. Moorehead). Chest: Increased in anteroposterior diameter. Intercostal spaces widened. Posture (sitting in bed) stooped. Lower lung borders anteriorly in midclavicular line at the seventh intercostal space; posteriorly, at the level of the twelfth spinous process. Excursion on deepest inspiration scarcely one-half inch. Vocal fremitus not increased. Percussion note hyperresonant over both lungs. Both inspiratory and expiratory phases markedly prolonged. Sonorous and sibilant râles over whole chest. Heart: Apex beat not visible or palpable. Epigastric pulsation present. Heart borders not determinable. All heart sounds soft and feeble but clear. No murmurs. Liver: Easily palpable and extends about 2 cm. below costal arch. Spleen: Palpable. No masses in the abdomen. Sputum: Bacteriologic examination done at several periods of her stay in the hospital showed aerobic: *Streptococcus viridans; Micrococcus catarrhalis* and *pneumococcus.* Anaerobic: *Bacillus annuliformis; Streptococcus parvus; Bacillus fusiformis.*

Treatment and Course.—Epinephrin, atropin, potassium iodid and strong coffee gave symptomatic relief of short duration. The administration of autogenous vaccines was followed by short periods of improvement. This, however, was not permanent and the recurrence of several paroxysms of dyspnea depressed the patient to such a degree of despondency that she sought and found a voluntary death through drowning. The body was in the water about three hours and was examined five hours after death.

Macroscopic Examination.—Not obtainable. (Coroner's case.)

Microscopic Examination.—The pleurae of both lungs vary in thickness and contain but a few foci of round cells. Most of the alveoli are of normal size, contain only an occasional endothelial cell, and have thin walls which also contain a few small foci of round cells. In the outer borders of both lungs the alveoli are dilated and some have ruptured walls. One small group of alveoli in the right lower lobe contains a fine granular precipitate in which there are numerous polymorphonuclear neutrophilic leukocytes. The ductuli respiratorii are patent, slightly dilated and their walls without appreciable change. The bronchioles, even to the smallest, are somewhat dilated and most of them are occluded by homogenous mucous masses which contain a few epithelial cells, round cells and many polymorphonuclear cells, many of which are eosinophilic. The walls are thin because of the dilatation, contain only a few round cells and have thin strands of smooth muscle fibers and of elastic tissue. The epithelium is only partially retained and in all except the smallest bronchioles it rests on a very prominent hyalinized basement membrane. Many bronchioli are dilated to such a degree that they can be differentiated from small bronchi only by their structure.

The small and middle sized bronchi are dilated so that longitudinal folds are obliterated, and almost all are occluded by mucous masses which vary somewhat in structure and constituents. In some, the mass is homogenous

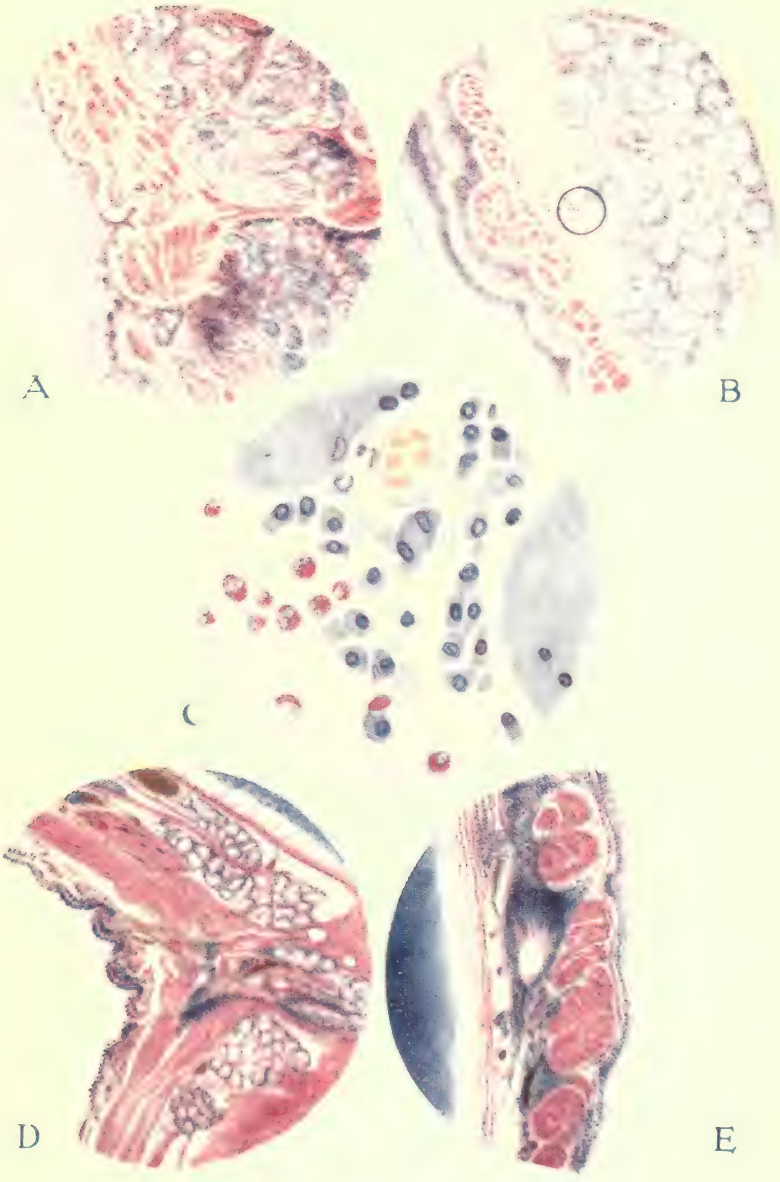

PLATE 1

Plate 1. Bacterial type of asthma. Case 1. A. Cross section of a large bronchus. Note the muscle bundles, basement membrane, marked hypertrophy of mucous glands, and cellular infiltration.

B. Longitudinal section of a large bronchus. Note the muscle bundles, cellular infiltration, mucous glands, and eosinophil cells. High magnification of the area marked by the circle is shown in C.

C. High magnification of the area marked by the circle in B.

D. Bacterial type of asthma. Case 2. Cross section of a large bronchus. Note the muscle bundles, mucous glands, and distended capillaries.

E. Longitudinal section of a large bronchus. Note the muscle bundles, distended capillaries and cellular infiltration.

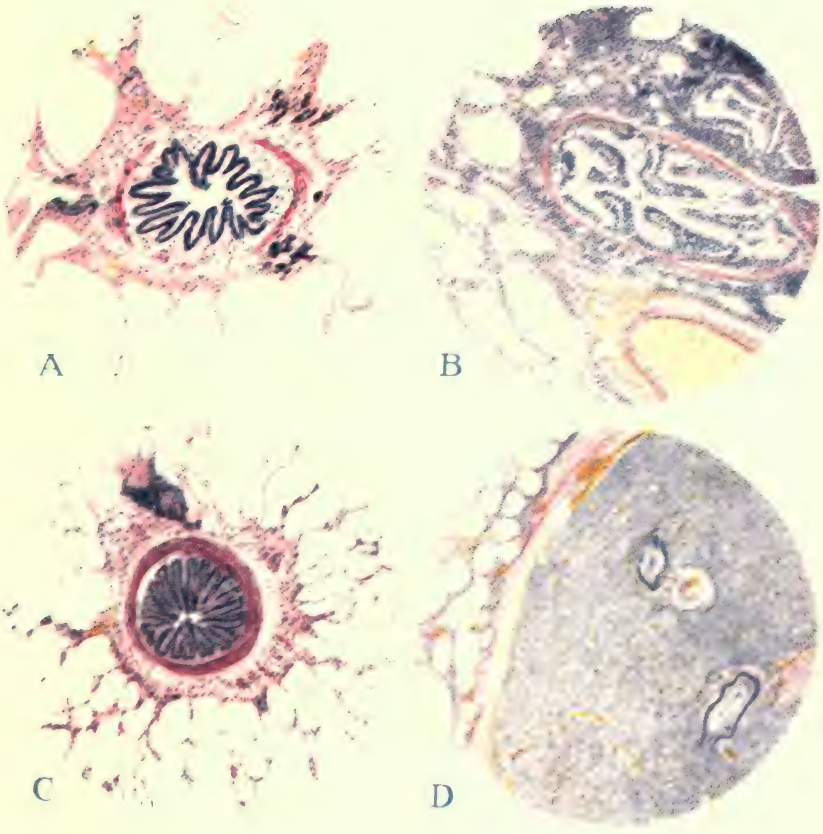

PLATE 2

Plate 2.—Bacterial type of asthma. Case 2. A. Bronchiole almost occluded by a folding of the epithelium.

B. Case 3. Bronchiole occluded by folds of epithelium.

C. Bronchiole of a guinea-pig. Fatal horse serum anaphylaxis.

D. Food asthma. Case 6. Area of absorption atelectasis with an adjoining area of normal lung tissue.

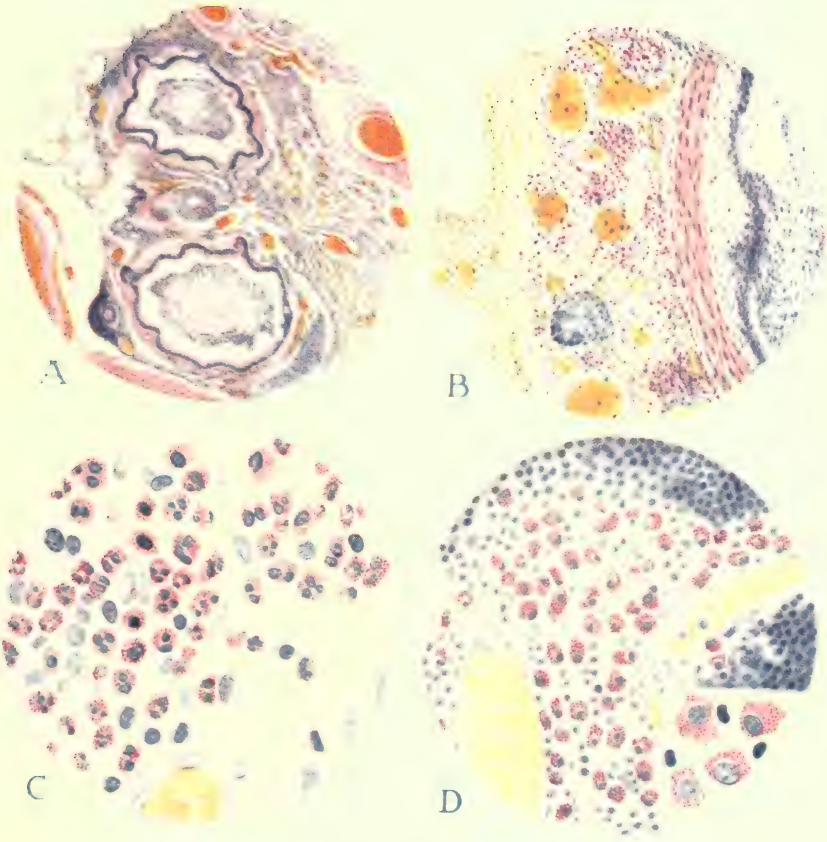

PLATE 3

Plate 3.—Food asthma. Case 6. A. Occlusion of bronchi by mucous exudate.

B. Cross section of a large bronchus. Note the muscle bundles, distended capillaries and extensive infiltration by eosinophil cells.

C. High magnification of an area of eosinophilic infiltration in the bronchial wall.

D. Mononuclear eosinophil cells in the thymus. Insert: a high magnification of these cells.

and contains diffusely scattered and polymorphonuclear cells which are mostly eosinophilic, some round cells and near the outer zone desquamated epithelial cells. In others, the mass appears much twisted, having whorllike thickened lines and rows of cells intermingled with layers of homogeneous cell free mucus. In many bronchi the mucous masses appear to be flowing stream-like into the bronchi from the gland ducts. No fibrin and only a few erythrocytes are found. The epithelium is for the most part desquamated, only the lower layer of small cells remaining attached to the thickened prominent basement membrane. The basement membrane is conspicuous and extends as a prominent band up into the gland ducts, at times even up to the gland itself. The subepithelial layer contains many cells chiefly small round cells, a few eosinophil cells, mostly polymorphonuclear, and a few mast cells. The elastic tissue is prominent in some bronchi and in all, the capillaries are numerous but small. The muscle tissue varies greatly in thickness, being prominent in all bronchi but in some forming large bundles which are best seen in the longitudinal sections (Fig. 2). There is no evidence of hyaline degeneration or fatty infiltration of the muscle. The mucous glands are very large and many are densely infiltrated by cells (Fig. 3). In most the acini are large, filled by mucus and are often separated widely by infiltrating cells which obscure large portions of the gland structure. These cells are mostly small round cells, but in some glands the predominating cells are polymorphonuclear eosinophil cells. Some glands are almost completely destroyed by this infiltration. The gland ducts leading from these glands are large and in many places distinct ampullae are formed which contain mucus or mucus inclosing many cells. Many of the ducts are surrounded by a zone of round cells (Plate 1, A, B, C). Some of the cartilaginous plaques in both lungs show small areas of calcification. The walls of some of the bronchial arteries are thickened. No nerves or nerve ganglia are invaded by round cells.

The structure of the larger bronchi is similar to that of the smaller bronchi except that the mucus does not completely occlude any of them. No Charcot-Leyden's crystals or Curshmann's spirals are found, and although the epithelium is missing throughout, no evidence of true ulceration can be demonstrated.

The bronchial lymph glands of both lungs are large and anthracotic; their sinuses contain a few polymorphonuclear eosinophils and they contain no evidence of tuberculosis.

Summary.—The most significant pulmonary pathologic findings in this case are: (1) Complete occlusion of the majority of the middle sized and small bronchi and of the bronchioles by a mucous cellular exudate. (2) Numerous large mucus-filled gland-duct ampullae (Schleimhautausstulpungen of Mönckeberg). (3) Large active mucous glands. (4) Marked cellular infiltration of the subepithelial layer and of the mucous glands. (5) Prominence of the basement membrane and of the muscular layer. (6) Tissue eosinophilia.

Comment. The history of previous attacks of bronchitis and of pneumonia, of asthmatic attacks beginning after the fiftieth year, and the microscopic evidence of infection in the bronchial walls point to bacteria as the causal agent in this case of asthma.

Case 2. Mr. G., aged 55, married, American, entered the Presbyterian Hospital, July, 1917, under the care of one of us (K. L.).

History.—Except for having had the usual diseases of childhood, he was exceptionally well until his forty-ninth summer (1911), when a severe bronchitis developed and persisted for six months, being accompanied near the end by wheezing sounds and one definite attack of spasmodic dyspnea. The following spring he had a second attack of bronchitis accompanied by wheezing sounds and one attack of spasmodic dyspnea. Following this the attacks

came more frequently each year so that, at the time of entrance into the hospital, six years after the first attack, they occurred as frequently as from three to five times daily with only short periods of freedom. During this time (six years) the weight gradually fell from 155 to 90 pounds. Tobacco and alcohol never used. Gonorrhea and syphilis denied.

During the three years prior to July, 1917, the following surgical and medical procedures were instituted without giving relief. 1914: Nasal polypi removed. 1915: Twenty injections of a stock vaccine. Potassium iodid up to 50 minims, three times daily. Teeth were roentgenographed and two were removed. Polypi and turbinates removed. 1916: Tonsils removed. Eleven injections of arsphenamin (negative blood Wassermann!). 1917: Use of mixed

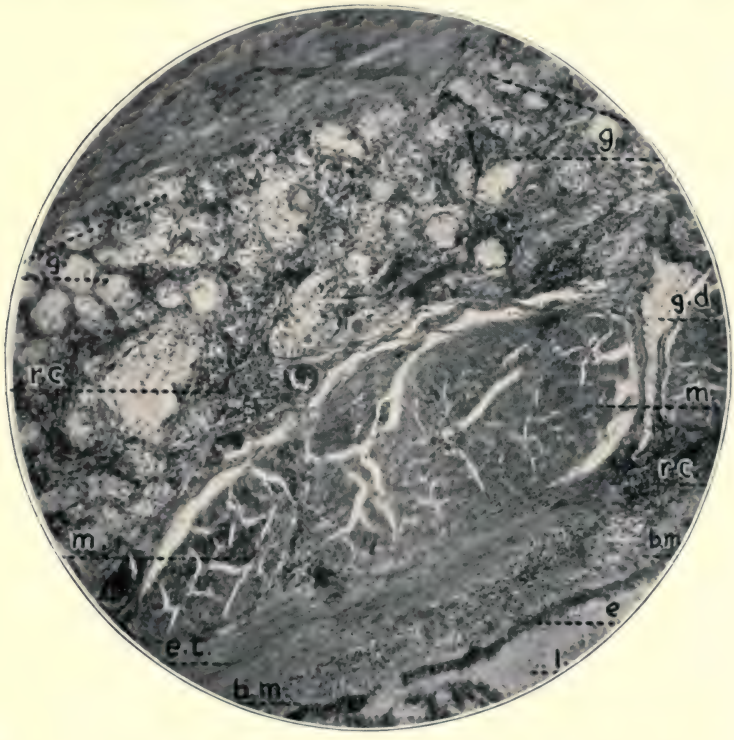

Fig. 2. Case 1. Bacterial asthma. Large bronchus. Longitudinal section. Epithelium intact. Basement membrane thick and hyalinized. Subepithelial layer infiltrated by round cells. Muscle bundles very large. Glands have distended acini and contain foci of round cells. l = lumen; e = epithelium; $b\,m$ = basement membrane; $e\,t$ = elastic tissue; $r\,c$ = round cells; m = muscle; $g\,d$ = gland duct; g = gland. Magnification, 65 diameters.

vaccine. Removal of colon was advised by family physician as roentgen-ray examination showed a viceroptosis, especially marked of the transverse colon. The patient had noted that frequent catharsis and use of enemas seemed to alleviate the attacks.

Family History.—Mother, two sisters, one brother, and one child have had asthma.

Physical Examination.—At the time of entrance into the hospital in the summer of 1917, the following observations were made: Small, much emaciated

man. Head and neck, negative. Chest: thin walled, expansion fair, numerous squeaky and whistling râles on both sides. Heart: Left border 10 cm. from midsternal line. Rate, tone and rhythm appear normal. Abdomen: Wall muscular. Small umbilical hernia. Liver: Palpable 2 cm. below costal margin in midclavicular line. Spleen: Not palpable. Inguinal glands: Easily palpable. Extremities: Negative.

Blood: Hemoglobin, 75 per cent.; leukocytes, 7,900; red cells, 3,670,000. Differential count: Small lymphocytes, 23 per cent.; large lymphocytes, 8 per cent.; polymorphonuclear neutrophils, 59 per cent.; polymorphonuclear eosinophils, 10 per cent.

Urine: Specific gravity, 1.032; no albumin, casts, sugar or pus.

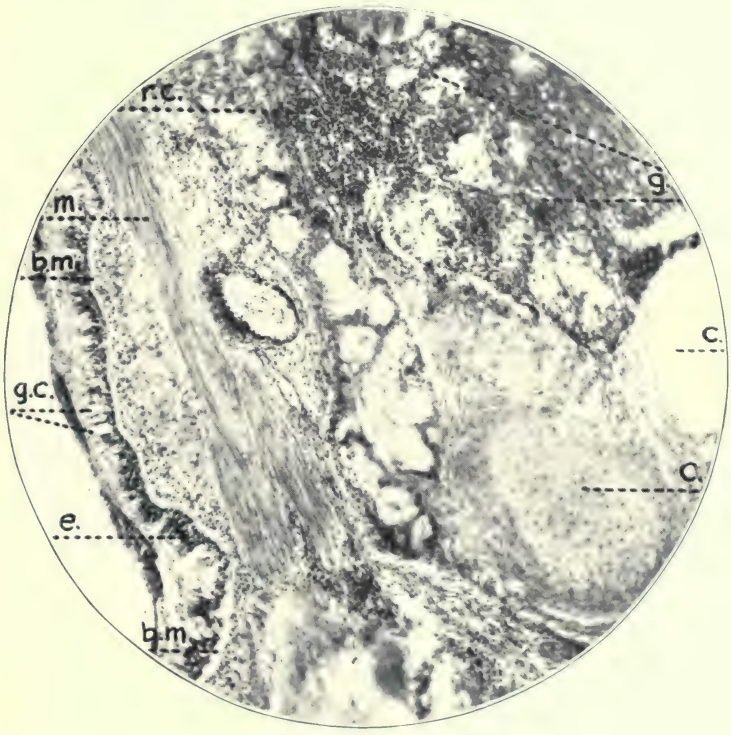

Fig. 3. Case 1. Bacterial asthma. Large bronchus. Cross section. Epithelium intact and containing goblet cells. Basement membrane thick and hyalinized. Subepithelial layer infiltrated by round cells. Muscle bundles large. Mucous glands large and infiltrated by round cells. e = epithelium; $g\,c$ = goblet cells; $b\,m$ = basement membrane; m = muscle; $r\,c$ = round cells; g = gland; c = cartilage. Magnification, 70 diameters.

Gastric content: Ewald test meal: Free acid, 8 per cent.; combined acid, 37 per cent.; total acidity, 45 per cent.

Electrocardiogram: Slight hyperbalance of right heart.

Blood pressure: 122/75.

Medication.—Potassium iodid, epinephrin and autogenous vaccine. At the time of entrance to the hospital the patient suffered from two to four attacks of dyspnea daily, coughed a great deal, raised a small amount of sticky mucus and complained of pain in chest and of abdominal distention and distress.

Ten minims of epinephrin usually gave prompt relief. However, after enjoying a period of quiescence for ten days, the patient died in an attack which had lasted three days. The necropsy was performed the next day.

Necropsy Report: *Anatomic Diagnosis.*—Chronic fibrous myocarditis; marked emphysema of the lungs; barrel-shaped chest; hyperemia of the pericardial sac; passive hyperemia of the liver and spleen; slight chronic interstitial nephritis and hyperemia of the kidneys; fibrous episplenitis; calvities and canities.

Macroscopic Examination.[20]—The body is that of a white man about 55 years of age, weighing approximately 100 pounds. Rigor mortis is marked and posterior lividity is present. Muscular development is fair and the bones are of medium size. The chest wall is bulging, and the abdomen flat. The subcutaneous fat in the midline of the trunk in front has a maximum thickness of 5 mm. The inguinal rings are closed. The liver extends two finger breadths below the costal margin. The diaphragm on the right side extends upward to the middle of the fourth rib, on the left to the fifth interspace. Thorax: The pericardial sac is blue, caused by the engorgement of the vessels which supply it. There is only the normal amount of fluid present. The apex of the heart is made up of the right ventricle. The pulmonary artery and the right heart contain only fluid blood. The wall of the left ventricle at its thickest portion measures 2 cm.; the right, 7 mm. It weighs 320 gm. The heart muscle is pale red and slightly fibrous. The lungs are huge and are free from adhesions. They are red and black in color and bulge out when the sternum is removed in the usual manner. The cut sections are spongy and some blood flows from their surfaces. Liver: The liver is reddish brown and weighs 1,450 gm. Its capsule is smooth, except in places where it has been adherent to the diaphragm. There is a yellow scar on the right lobe 8 mm. in its largest diameter, which when cut does not extend into the liver substance. The cut surfaces are red and exude much blood. Kidneys: The kidneys together weigh 230 gm. Their capsules are smooth, but strip with difficulty, leaving a rough irregular surface. The cut surfaces are red and the cortical markings are fairly distinct. Spleen: The spleen is rough and slaty blue, except the rough surface made up of yellowish nodules averaging 2 mm. in diameter. The cut surfaces are very red and pulpy, much blood flowing from these surfaces. It weighs 150 gm.

Microscopic Examination.—The pleura varies in thickness from 0.06 to 0.09 mm., and shows no abnormalities except a few widely distended blood vessels. The alveoli are not abnormally distended, except in patches in the periphery of the upper lobes of both lungs and they are empty, except in the left lower lobe where a few small areas are obliterated by accumulations of a fine granular precipitate which contains only a few erythrocytes. The alveolar walls throughout both lungs are thickened by distention of capillaries and an occasional accumulation of small mononuclear cells. The epithelium of the ductuli respiratorii is intact and shows no abnormalities. The walls of these passages contain short muscle bundles from 0.010 to 0.015 mm. in thickness and only a few small mononuclear cells.

The bronchioles in all parts of the lungs are similar in structure. In a few of the smaller bronchioles the lumina are narrowed by a deep folding of the epithelium (Plate 2 A). In a few others the lumina are occluded by a fine granular precipitate containing only a few large pigmented mononuclear cells, while in the majority the lumina are patent and contain only a few of the pigmented cells. The epithelium is well perserved, is thrown into longitudinal folds varying in height from 0.010 to 0.085 mm. and having a thickness in the smaller bronchioles of from 0.0075 to 0.020 mm. and in the larger bronchioles from 0.050 to 0.095 mm. No goblet cells are visible. The basement membrane is not conspicuous. The subepithelial layer is thin and contains only a very few

20. Only those parts of the complete necropsy reports which seemed to have a possible relationship to the problem of our study are given here.

mononuclear cells. The muscle layer, varying in thickness from thin strands to definite bundles 0.060 mm. thick, does not form a continuous layer and varies greatly in size in different parts of the circumference of the bronchiole. The elastic tissue is scanty and the fibrous tissue is found only in moderate amounts.

The structure of the bronchi in all parts of the lungs is essentially the same. The lumina of all contain only a small amount of granular precipitate in which there are fragments of desquamated epithelium. The epithelium is thrown into longitudinal folds from 0.06 to 0.24 mm. high and contains only a few goblet cells. There is no evidence of new or old ulcer formation in the epithelium. The basement membrane is visible only in parts of the circumference. The subepithelial layer is thin and contains distended capillaries, scattered small mononuclear cells, a few polymorphonuclear eosinophil cells, plasma cells and small bundles of elastic tissue. The muscle layer, which in many bronchi lies unusually near the epithelium, is very well developed, the bundles in some of the larger bronchi being 0.23 mm. in thickness (Fig. 4). These bundles, as seen best in the longitudinal sections, are separated from each other by only a thin connective tissue layer containing distended capillaries (Plate 1 D, and E). The mucous glands are of moderate size and their acini are small. Most of the glands contain dense foci of small mononuclear cells, and in many of them only small fragments of gland tissue remain. The outer portions of many of the glands contain many fat cells. Many of the gland ducts in the subepithelial layer are surrounded by a wide zone of closely placed small mononuclear cells, and in many ducts the lumina form conspicuous ampullae-like enlargements between the cartilage and muscle layer. These ampullae-like structures contain a small amount of mucus in which there is much desquamated epithelium. The cartilages are unchanged. The distended capillaries in all layers of the bronchi are very conspicuous and show well their distribution in these structures. The walls of many of the bronchial arteries in the larger bronchi are very thick. The ganglia, nerves and nerve sheaths are not infiltrated or surrounded by cells.

Comment.—The most significant pathologic changes in this case are: (1) The numerous large gland duct ampullae. (2) The extensive cellular infiltration in the bronchial mucous glands. (3) Small size of bronchial mucous glands and abundant periglandular collections of fat cells. (4) Prominence of the bronchial muscular system. (5) Thickening of the walls of the bronchial arteries. The history of a severe bronchitis preceding and accompanying the attacks of dyspnea, of asthmatic attacks beginning about the fiftieth year and the microscopic evidence of infection of the bronchial mucous glands point to bacteria as the causal agents in this case of asthma.

Case 3.—Miss L., aged 17, entered the Cook County Hospital suffering from an acute infection.

History.—Since early childhood she had had frequent severe attacks of spasmodic dyspnea which were always worse in summer and were accompanied by severe coughing and raising of a thick sticky sputum. The attacks, while less frequent in the past year, had become more severe in character.

Family History.—Negative.

Examination.—Pulse, 116; respiration, 28; temperature 99.4 F.; blood pressure, 110/72. Blood: Hemoglobin, 75 per cent.; leukocytes, 17,200; red blood cells, 4,420,000. Differential count: small lymphocytes, 8 per cent.; large lymphocytes, 2 per cent.; neutrophils, 84 per cent.; eosinophils, 5 per cent.;

transitionals, 1 per cent. Marked pyorrhea alveolaris; bilateral cervical adenopathy.

Urine: Positive for albumen and casts. Thorax: increased anteroposterior diameter; normal lung borders; diminished excursion on both sides; percussion note hyperresonant over whole chest; numerous wheezing and squeaking râles on both sides; one small area of crackling râles over the medium dependent portion of the left lower lobe. Heart: Heart tones indistinctly heard; no murmurs. Abdomen: Negative. Adenopathy of axillary and inguinal glands. Reflexes, normal.

Diagnosis.—Bronchial asthma; localized patch of bronchitis; emphysema; nephritis and pyorrhea alveolaris.

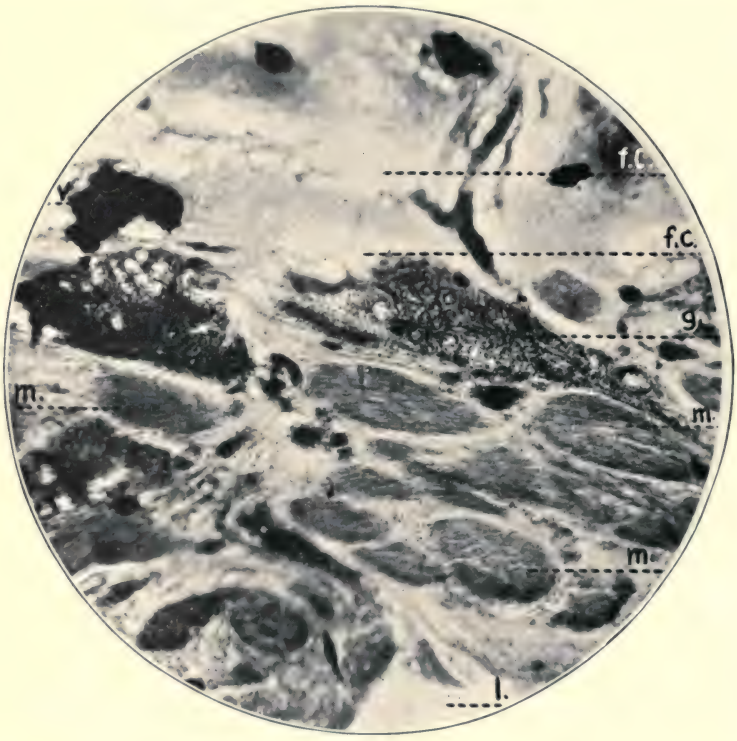

Fig. 4.—Case 2. Bacterial asthma. Large bronchus. Cross section. Epithelium desquamated. Basement membrane not visible. Subepithelial layer contains few cells. Muscle bundles large. Glands small and contain foci of round cells. All vessels distended. l = lumen; m = muscle; g = gland; $f\,c$ = fat cells; v = vein. Magnification, 45 diameters.

Course.—There was a steady improvement in condition for sixteen days, then a sore throat developed, with abdominal pain, vomiting and other symptoms of acute intoxication. The patient died three days later.

Necropsy Report (Dr. Stangl): *Anatomic Diagnosis.*—Acute hemolytic streptococcus pericarditis and peritonitis; cloudy swelling of myocardium, liver and kidneys; acute emaciation; fibrous obliteration of the right pleural cavity; hyperplasia of the spleen. Bacterial cultures of pericardial fluid and heart blood showed hemolytic streptococci.

Macroscopic Examination.—The body is that of a small, poorly nourished white girl about 12 years of age. Height, 146 cm. The lips and fingers are cynotic. The thyroid is slightly enlarged. The skeletal muscles are well developed. The axillary lymph glands are somewhat enlarged. Abdomen: The abdominal cavity contains a large quantity of a brownish fluid in which are flocculi of fibrin. The omentum hangs low and is covered with fibrin. The peritoneal surface of the intestines is reddened and coated with fibrin. The lower border of the liver is at the costal margin. There are fibrous adhesions between the fundus of the gallbladder and the stomach and duodenum. The diaphragm reaches to the fifth rib on the right side and to the fourth rib on the left. Pleural cavity: The right pleural cavity is entirely obliterated by fibrous adhesions. The left cavity is free of adhesions. There

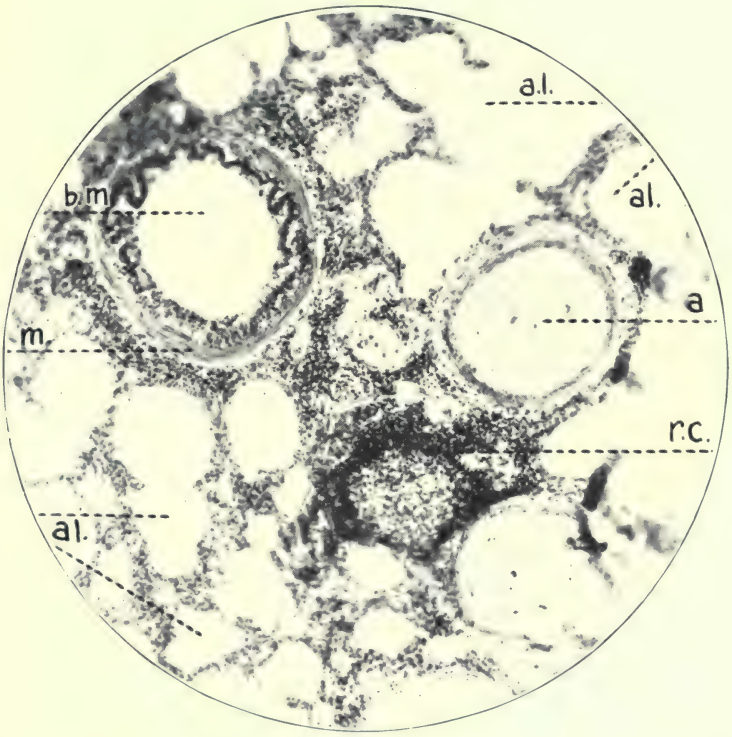

Fig. 5.—Case 3. Food (?) asthma. Bronchiole. Cross section. Muscle layer prominent. Area of round cells. *b r* = bronchiole; *m* = muscle; *a* = artery; *al* = alveoli; *r c* = round cells. Magnification, 85 diameters.

is a moderate amount of granulation tissue at the site of the thymus. Pericardial cavity: The pericardial cavity contains a small amount of turbid fluid in which there is fibrin. There are many petechial hemorrhages beneath the epicardium. Heart: The venae cavae are engorged with dark clotted blood. The tricuspid and aortic valves are normal. There is a healed vegetative growth on one cusp of the mitral valve. The myocardium is pale red and grossly without change. The heart weighs 400 gm. Lungs: The surface of the right lung is covered by extensive fibrous adhesions, but it crepitates everywhere and is free from coal dust pigmentation. It weighs 320 gm. The surface of the left lung is smooth and it crepitates everywhere. The trachea and

bronchi exude a thick mucopus and the mucosa is hyperemic. The tracheobronchial lymph glands are brown in color. Liver: The liver is mottled reddish brown and yellow and its capsule is smooth and dull. Spleen: Large and firm and weighs 320 gm. The solitary lymph follicles of the large intestine are prominent (2 mm.), and some are surrounded by small hemorrhages. There is one small cyst in each ovary. Kidneys: Weight, 400 gm.; mottled in color; fetal lobulations. The capsule is smooth and strips readily, leaving a smooth surface. The cortical striations are plainly visible. The lining of the pelvis is smooth and unchanged. Head: The brain and meninges show no gross changes.

Microscopic Examination.—Left lung examined. The pleura is slightly thickened throughout and contains numerous small foci of small round cells. Most of the alveoli are small, containing only a few pigmented endothelial cells, and their walls are considerably thickened by dilated capillaries, and numerous small accumulations of small round cells. The remaining alveoli are moderately dilated, contain no exudate and have thinner walls.

The bronchioles in the whole lung vary markedly in size and structure, some being dilated and with thin walls, others being markedly contracted. In some the folds of epithelium have sloughed off and completely occluded the lumen (Plate 2 B). The lumina of the former vary in size from 0.25 to 0.5 mm. and the latter from 0.07 to 0.15 mm. The other measurements are given in Table 1. The single layered low cuboidal epithelium is intact and the subepithelial layer contains a few small round cells, a small amount of elastic tissue and an occasional polynuclear eosinophil cell. The muscular layer in some bronchioles form a distinct complete circular ring but in most it is interrupted (Fig. 5).

The lumina of the smaller and medium sized bronchi contain only a small amount of exudate which adheres to the epithelium and consists chiefly of mucus containing epithelial cells either isolated or in clumps. Among these cells there are a few polymorphonuclear neutrophils and eosinophils and a few round cells. The epithelium is intact except in the deeper cryptlike pockets between folds where only the lower layer of cells remains. The outer layer of the epithelium contains numerous goblet cells and in some bronchi there are a few small round cells and polynuclear eosinophil cells between the epithelial cells. There is no appreciable lengthening of the epithelial cells in any portions of the bronchi. The basement membrane is distinct and appears hyalin even in the bronchioles.

The subepithelial layer varies considerably in thickness even in the same bronchus, as is well shown by the longitudinal sections. This layer, especially that portion lying nearest the basement membrane, is infiltrated by numerous small round cells, plasma cells and a few polymorphonuclear eosinophil cells. The longitudinal and circular bundles of elastic tissue are not increased in size and the capillaries are not distended.

The muscular layer in all bronchi is well developed and shows especially in the longitudinal sections the distinct bundle arrangement (Fig. 6). The connective tissue separating and extending into these bundles contains a few polymorphonuclear eosinophil cells and a few small round cells.

The mucous glands which are found in all bronchi having an outside diameter greater than 2 mm. are large and most are infiltrated extensively by small round cells. The acini of the glands are not in active secretion and in many glands they are distorted and widely separated by the infiltrating round cells, while in some glands only a few small acini remain. The walls of the mucous-gland ducts contain many round cells and the tissue around many ducts is extensively infiltrated. Many of these gland ducts are widely dilated and form distinct ampullae containing mucus, desquamated epithelium and round cells. No eosinophil cells are found in any of the glands.

Cartilages are found in smaller divisions of the bronchi than the glands and many contain small calcified areas. One cartilage in a small bronchus with an outside diameter of 2.8 mm. contains a small ossified portion with a true marrow center (Fig. 7).

The walls of the bronchial arteries are moderately thickened and the nerves and ganglia are surrounded by connective tissue containing only a few round cells.

The content and walls of the larger bronchi are similar to that in the smaller bronchi, except that the goblet cells are somewhat more numerous in the former. There is no essential difference in findings in the bronchi of the upper and the lower lobes. Measurements of the bronchi will be found in Table 2.

Comment.—The most significant pulmonary pathologic findings in this case are: (1) Right sided adhesive pleuritis. (2) Extensive

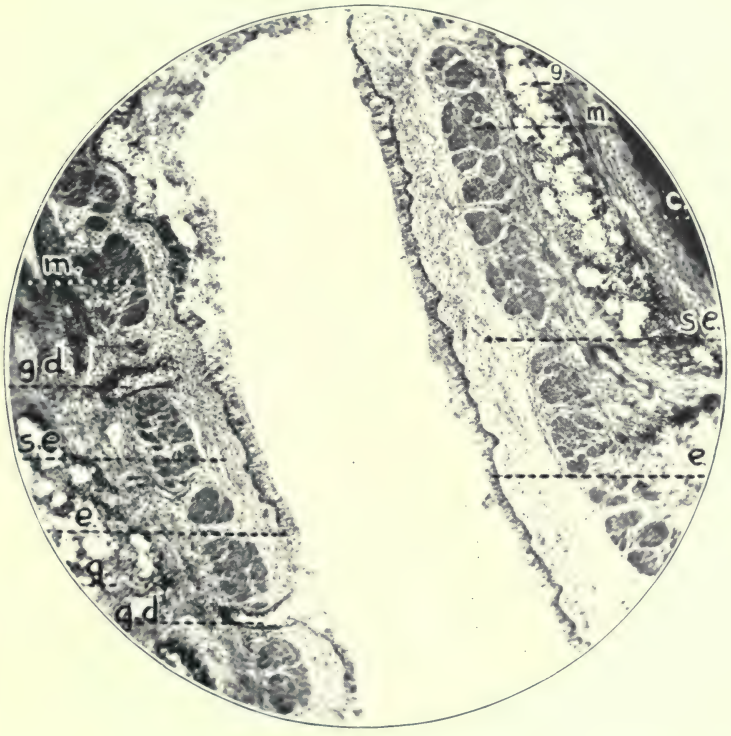

Fig. 6.—Case 3. Food (?) asthma. Small bronchus. Longitudinal section. Epithelium shows goblet cells. Basement membrane prominent. Subepithelial layer infiltrated by round cells. Muscle bundles prominent. Glands contain round cells. e = epithelium; $g\,c$ = goblet cells; $s\,e$ = subepithelial layer; m = muscle; $g\,d$ = gland duct; g = gland; c = cartilage. Magnification, 45 diameters.

cellular infiltration of the subepithelial layer, the mucous glands, and the walls of mucous-gland ducts. (3) Numerous ampullae-like gland ducts. (4) Prominence of muscle layer. (5) Calcification and ossification of cartilages. (6) Eosinophilic infiltration of bronchial wall.

The history of asthma since childhood suggests the probability of sensitization to plant or animal proteins. This point cannot be determined definitely, as no skin sensitization tests were made and a complete clinical

history was not obtainable. The evidence of an old infection, the adhesive pleuritis, and the evidence of present infection in the mucous glands and the subepithelial layer of the bronchi point to bacteria as secondary agents in this case of asthma.

Case 4.—Mr. E. L., aged 29.

History.—The complete history is not available. Patient had had frequent head colds and asthmatic attacks since the age of 10 and was unable to do any work until his twenty-seventh year when, following medication and use of an autogenous vaccine there was a period of improvement.

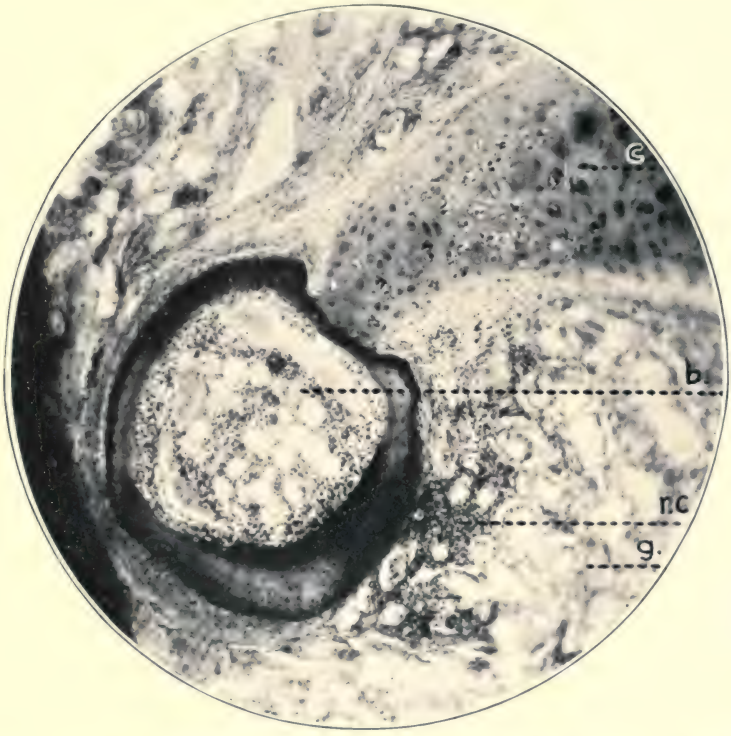

Fig. 7.—Case 3. Food (?) asthma. Bronchial cartilage with ossification. $b =$ bone with typical bone marrow; $r\,c =$ round cells; $g =$ gland; $c =$ cartilage. Magnification, 70 diameters.

Family History.—Mother had asthma after thirty-fifth year.

Course.—While in the army he discovered that proximity to horses or mules precipitated attacks of asthma and he therefore wished to be desensitized. This was attempted by his physician and death occurred within ten minutes after an intravenous injection of 1 minim of horse serum. Here we need give only a few points of particular interest, as the complete report of the necropsy findings is given by Boughton.[21] He says in part:

21. Boughton, T. H.: Anaphylactic Death in Asthmatics, J. A. M. A. **73**: 1912 (Dec. 29) 1919.

Both lungs were enormously distended and emphysematous. The left lung showed a small area of hemorrhage on the lateral portion of the lower lobe, about 4 cm. in diameter, with a gelatinous organizing exudate at this point. On section the lungs are dry. The right pleural cavity was largely obliterated by firm fibrous adhesions. No fluid was present in either cavity. The heart was firm and of normal size and appearance except for a few subpericardial petechial hemorrhages on the posterior surface.

Microscopic Examination.—Lungs: There was a moderate passive hyperemia, but no edema. There were a few small interstitial hemorrhages. A little mucus and a few desquamated epithelial cells were seen in some of the bronchioles. The peribronchiolar muscle was well developed. A few arteries showed greatly thickened walls, and many showed a moderate thickening of the walls. Eosinophils were very numerous in the spleen.

Microscopic Examination.—Drs. Boughton and Raulston, the pathologists, kindly furnished us with the blocks of tissue from this case so that we could make a more detailed study of the lungs. Our findings are as follows: The structure of the pleura varies from a thin layer 0.09 mm. thick with no appreciable changes to a layer 1.35 mm. thick. This increased thickness is due chiefly to fibrous tissue, the outer layer of which is less compact and contains numerous small extravasations of erythrocytes. The pleura, especially in the thickened portion, contains numerous small capillaries, scattered small round cells, a few small foci of round cells, but no eosinophil cells. In some obliterated interlobar spaces and in portions of thickened pleura, where adhesions are evident, small thick-walled arteries are very conspicuous. The alveoli have no uniformity in size due to the unusual variation in structure of the interstitial tissue. Some are widely dilated and have thin intact or ruptured walls while those adjoining may be small and have walls thickened by fibrous tissue and cellular infiltration. The lumina of most of the alveoli contain only an occasional large pigmented mononuclear cell, but in a few small areas they are completely filled by a finely granular precipitate containing an occasional erythrocyte. The patchy thickening of the interstitial tissue is very striking and in many parts of the lungs patches of alveoli are almost completely obliterated by a dense tissue which consists of a network of fibrous tissue surrounding various sized groups of cells. These cells are chiefly small round cells, often grouped, with densely stained nuclei and scanty cytoplasm, large cells with small nuclei and abundant homogeneous cytoplasm, plasma cells, pigmented endothelial cells, a few polymorphonuclear neutrophil cells and an occasional polymorphonuclear eosinophil cell. Throughout this tissue there are numerous fragments of hyperplastic epithelium, some small isolated bronchioles with walls that are densely infiltrated by small round cells, and which are surrounded by a thick zone of fibrous tissue, thin strands of nonstriated muscle fibers, and numerous small thick walled arteries surrounded by a thick zone of dense fibrous tissue.

The epithelium of the ductuli respiratorii is intact and the supporting layer of tissue contains numerous small round cells, a few polymorphonuclear eosinophil cells, and in many places short bundles of smooth muscle from 0.01 to 0.02 mm. thick.

The smallest bronchioles contain only an occasional pigmented mononuclear cell and their lumina are narrowed by longitudinal folds of epithelium from 0.03 to 0.04 mm. high. The epithelium is intact and contains no goblet-like cells. The basement membrane appears hyalin and is distinctly visible for only parts of the circumference. The subepithelial layer contains numerous small mononuclear cells, a few polymorphonuclear eosinophil cells, a few plasma cells, and a few constricted capillaries (epinephrin effect?). The muscular layer varies greatly in thickness in different bronchioles and does not form a continuous band. The outer layer of tissue is composed of loose fibrous tissue in which are many cells similar to those in the subepithelial layer.

The larger bronchioles are almost filled by a granular precipitate which contains only a few cells, chiefly pigmented mononuclear cells, a few neutrophil polymorphonuclears, and a few erythrocytes. The epithelium is from 0.027 to 0.05 mm. high and forms longitudinal folds from 0.12 to 0.18 mm. high. The portion of the epithelium nearest the lumen is made up of long ciliated

cells among which are numerous goblet-like cells. The deeper portions of the epithelium contain numerous small round cells. The other layers of the wall are similar to those of the smaller bronchioles except that the muscle layer forms larger bundles and the cellular infiltration is more marked. In the outer portion of the walls of many of these bronchioles there are masses of fibrous tissue from 0.21 to 0.50 mm. in thickness surrounding arteries with irregularly thickened walls. These vessels suggest the nodal arrangement of the bronchial arteries of guinea-pigs that have succumbed to anaphylactic shock[22] (Figs. 8, 9 and 10).

The lumina of the small bronchi contain only a small amount of a granular precipitate similar to that in the larger bronchioles. The epithelium, which is similar in structure to that in the larger bronchioles, is from 0.09 to

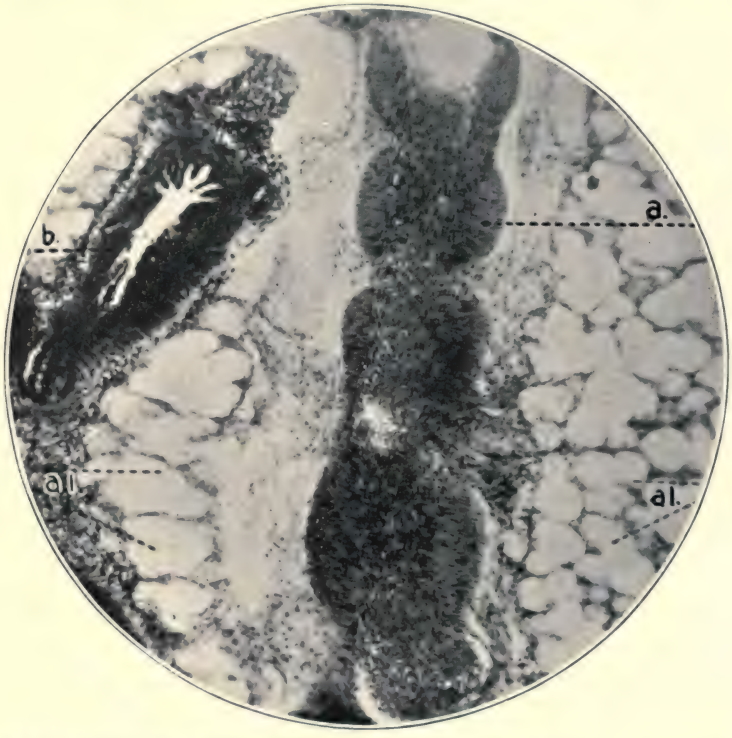

Fig. 8.—Guinea-pig. Fatal horse serum anaphylaxis. Artery. Nodular thickening of muscle. *a* = artery; *al* = alveoli; *b* = bronchiole. Magnification, 90 diameters.

0.12 mm. high and is thrown into folds from 0.20 to 0.27 mm. high. The basement membrane is thick and hyalin in appearance. The subepithelial and muscular layers are similar to those in the bronchioles but are markedly thicker. The mucous glands are almost completely obliterated by infiltration of small mononuclear cells, only a few atrophic acini remaining in most of the glands. The gland ducts are small and their walls are infiltrated by many

22. Schultz, W. H., and Jordan, H. E.: A Microscopic Study of the Anaphylactic Lung of the Guinea-Pig and Mouse, J. Pharmacol. & Exper. Therap. **2**:375, 1911.

small mononuclear cells. Throughout the tissues studied there is no evidence of new ulcer formation in the epithelium and no evidence of healed ulcers except possibly localized collections of small round cells in the subepithelial layers (Fig. 11).

The pulmonary and bronchial arteries and veins in many parts of the lungs are surrounded by varying amounts of dense fibrous tissue containing a few round cells and ranging in thickness from 0.15 to 0.5 mm. The media of many bronchial arteries is irregularly thickened and measures 0.18 mm. in some cases with a diameter of 0.45 mm. The walls of many smaller arteries contain areas of calcification. The spleen contains a large number of polymorphonuclear eosinophil cells.

The cartilages show some areas of calcification and one cartilage contains a small calcified area surrounding typical bone marrow.

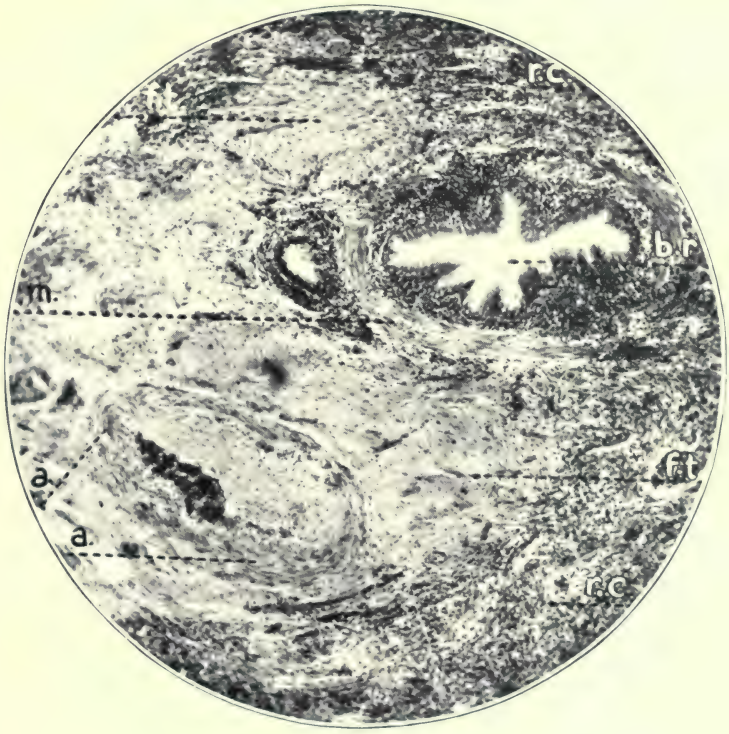

Fig. 9.—Case 4. Horse asthma. Fatal horse serum anaphylaxis. Artery and bronchiole surrounded by fibrous tissue. *a* = artery; *br* = bronchiole; *m* = muscle; *f t* = fibrous tissue. Magnification, 60 diameters.

Comment.—The most significant pathologic findings in this case are: (1) Right sided adhesive pleuritis. (2) Completely consolidated areas of pulmonary tissue. (3) marked cellular infiltration of the walls of the bronchi and bronchioles. (4) Moderate thickening of muscle tissue in the bronchi. (5) Atrophy of bronchial mucous glands. (6) Thickening of the walls of many pulmonary and bronchial arteries. (7) Wide zone of fibrous tissue surrounding many pulmonary and bronchial

arteries. (8) Eosinophilic infiltration in bronchial wall and in the spleen. (9) Calcification and ossification of bronchial cartilages.

The history of head colds and asthma since the tenth year with the subsequent discovery of the sensitiveness to horse dander leads one to believe that this case was from the beginning one of horse asthma. The adhesive pleuritis, the areas of consolidated lung tissue, and the evidence of extensive bronchial infection cannot be overlooked and the question naturally arises whether these factors are the result of the asthmatic attacks or are implicated in the production of the attacks.

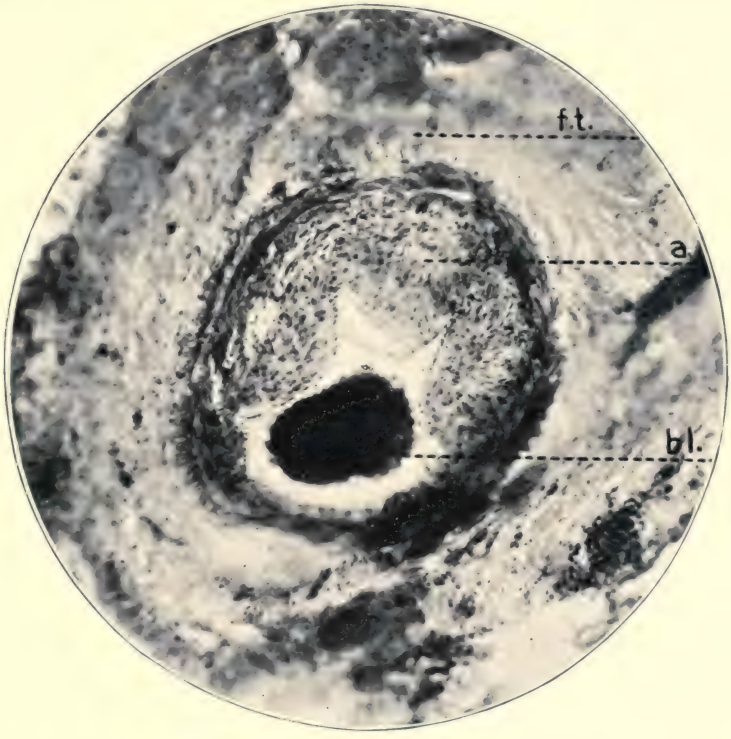

Fig. 10.—Case 4. Horse asthma. Fatal horse serum anaphylaxis. Artery surrounded by fibrous tissue and having irregularly thickened walls. *a* = artery; *bl* = blood; *f t* = fibrous tissue. Magnification, 100 diameters.

Case 5.—Mr. L., aged 32, single, American, car repairer.

History.—Previous illnesses: Measles, whooping cough and malaria. From childhood until going to California in his twenty-fourth year he suffered from frequent paroxysmal attacks of dyspnea. After a free period of seven years in California he returned to Chicago six months before death and suffered from a recurrence of the attacks. One week before death, April 4, 1912, he entered the Cook County Hospital suffering from bronchopneumonia.

Physical Examination.—Slightly built, white male. Head and eyes negative Chest: Supraclavicular fossae deep; epigastric angle wide; accessory respira-

tory muscles used during respiration, coarse sibilant râles heard posteriorly. Heart and abdomen negative. Urine contains erythrocytes. Sputum, no tubercle bacilli. Blood differential count: Neutrophils, 84 per cent.; large mononuclears, 8 per cent.; small mononuclears, 8 per cent. Temperature, 102 F. The condition of the patient gradually became worse and death occurred seven days after entrance. Postmortem examination by Dr. H. G. Wells.

Necropsy Report: *Anatomic Diagnosis.*—Acute, left, serofibrinous pleuritis; abscess in the lower lobe of the left lung; pulmonary emphysema; fibrinous pneumonia of the posterior portion of the left upper lobe; bronchopneumonia in right lung, chronic bronchitis; acute mediastinal lymphadenitis; slight hypertrophy of the right ventricle.

Macroscopic Examination.—External appearance: The body is that of a poorly nourished man; height 160 cm. The chest is very prominent anteroposteriorly; the costal cartilages bulge forward, particularly on the right side, and the intercostal spaces are considerably increased in width. The diaphragm reaches to the fifth interspace on the right side; to the sixth rib on the left side. Thorax: The right pleural cavity contains no fluid. The left cavity, however, contains considerable fluid which is turbid, blood stained, and full of fibrin. The serous membrane of the pericardium is smooth and shining, and shows no evidences of inflammation. Mouth: The pharynx is normal except for a slight edema about the arytenoids. Heart: Contracted and extremely rigid. Its size and proportions are apparently normal except for a slight hypertrophy of the right ventricle. Its weight is about 300 gm. The walls of the right ventricle are very muscular, averaging 4 mm. in thickness. The aortic, pulmonic, mitral and tricuspid valves all appear normal. Neither the aortic ring nor the thoracic aorta show any sclerosis. Lungs: The right lung is adherent anteriorly to the chest wall by weak fibrous adhesions, while its base is attached by firmer adhesions to the diaphragm. The lung itself shows no tendency to collapse and on being placed in water floats high. The substance of the lung itself is soft, pale, spongy and inelastic. Palpation reveals slight nodular and irregular hardenings. The pleura shows evidences of hemorrhage. The cut surface appears slightly raised, and mottled with the irregular hardened areas of bronchial pneumonia. Posteriorly this lung is full of fluid. The left lung, externally, appears light in color much as if it had been boiled. Like the right lung it does not collapse. The lower lobe is small, due to the compression of the fluid found in the left pleural cavity, and its lower portion contains much fluid. Its cut surface reveals prominent, thickened bronchi, and in one place a small abscess, 5 x 8 mm., which lies just beneath the pleura. Over this abscess the fibrin is much thicker and whiter than elsewhere. The upper lobe presents in its posterior portion a diffuse consolidation such as is seen in very wet, early pneumonia, such as is secondary to edema. The walls of the bronchi in this lobe are also thickened. There are no evidences of tuberculosis.

Peribronchial lymph glands: Are enlarged and reddened due to their proximity to the adjacent pleuritis. None caseous or calcified. Liver: Weight, 1,400 gm. The cut surface shows the lobules to be well marked and slightly darker at their periphery. Spleen: The spleen is of normal size, pinkish gray and soft. Gastro intestinal tract: In the lower portion of the ileum Peyer's patches show some slight pigmentation such as frequently follows typhoid.

Microscopic Examination.—Spleen: The malphigian corpuscles are small; increase in number of trabeculae; hyaline changes around the arteries. Liver: Shows slight passive hyperemia. Heart muscle: Slight increase in connective tissue. Lungs: There is an increase in the connective tissue around the arteries and bronchi. The alveolae are filled with desquamated cells and fibrin and some polymorphonuclears. Some of the alveolae show edema. Some areas show beginning abscesses. The mucous glands show hyperplasia. Kid-

ney: There is fibrosis of some of the glomeruli with round cell infiltration under the capsule. There are some albuminous casts in the tubules. Lymph glands: The glands show a great deal of anthracosis, some hyperplasia and some eosinophils.

Detailed Histologic Examination.—A systematic study of the lungs of this case was not possible as only portions of the lungs were preserved. Our findings are as follows: The pleura, with a fairly uniform thickness of from 0.075 to 0.15 mm. contains numerous distended capillaries, scattered small round cells, and a few small foci of round cells. The lung structure are obliterated in patches by pneumonic foci which are surrounded by alveoli containing a granular precipitate and in some areas extravasations of erythro-

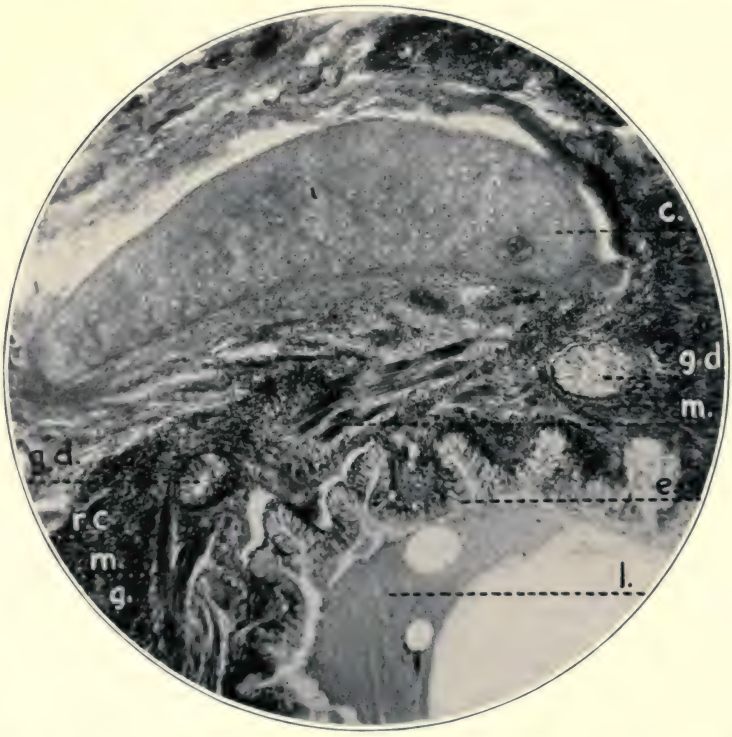

Fig. 11.—Case 4. Fatal horse serum anaphylaxis. Small bronchus. Cross section. Epithelium swollen. Subepithelial layer infiltrated by round cells. Glands small and infiltrated by round cells. l = lumen; e = epithelium; m = muscle; g = gland; $g\,d$ = gland duct; c = cartilage. Magnification, 45 diameters.

cytes. The alveolar walls in the areas not involved in the pneumonic process are not appreciably changed.

The lumina of the smaller bronchioles are partially occluded by fragments of desquamated epithelium, polymorphonuclear neutrophil cells, and a few erythrocytes. The single layered epithelium is partially desquamated and the basement membrane is indistinct. The subepithelial layer contains numerous small mononuclear cells, a few plasma cells, a few polymorphonuclear neutrophil cells and some distended capillaries. The muscle bundles are small and do not form a continuous layer. The larger bronchioles are similar to the

smaller, except that all layers are proportionately better developed. The elastic and fibrous tissues are very scanty in all the bronchioles.

The lumina of the small middle-sized bronchi contain the same character of material as that found in the bronchioles. The stratified epithelium is almost completely desquamated and the basement membrane is inconspicuous. The subepithelial layer is markedly thickened and contains many small mononuclear cells, a few polymorphonuclear neutrophil cells, occasional polymorphonuclear eosinophil cells, numerous distended capillaries and small bundles of elastic tissue. The smooth muscle layer is not prominent and its short bundles are separated and more or less obscured by fibrous tissue and invading small mononuclear cells. The cartilages show only a few small calcified areas.

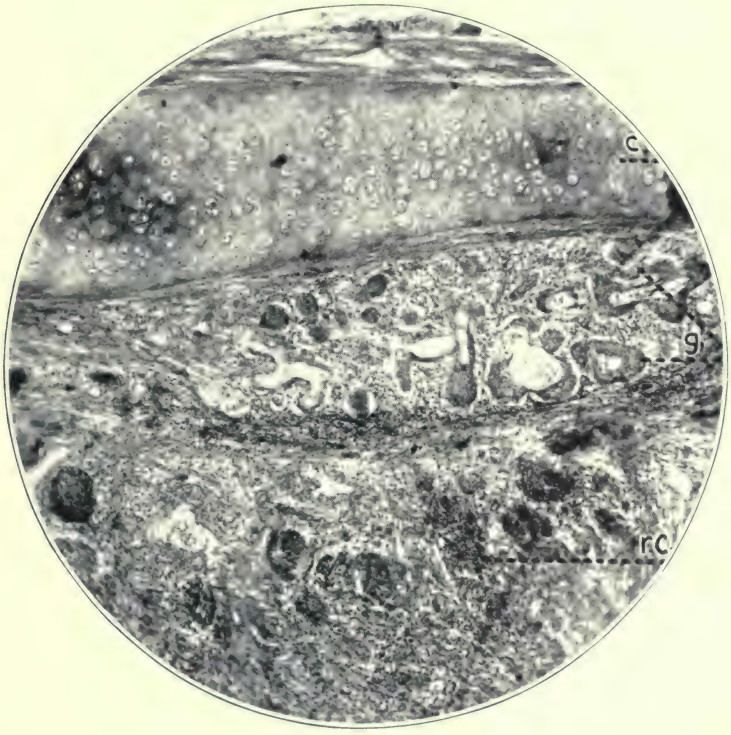

Fig. 12.—Case 5. Asthma. Bronchus. Cross section. Subepithelial layer markedly infiltrated by round cells. Mucous gland infiltrated and containing hyperplastic acinar cells. $r\,c$ = round cells; g = gland; c = cartilage. Magnification, 60 diameters.

Most of the mucous glands are small their acini are few in number, markedly distorted and separated by a cell rich tissue containing plasma cells, small mononuclear cells, a few polymorphonuclear neutrophil cells, a few mononuclear eosinophil cells, and a type of cell with a large pale nucleus and an abundant homogenous cytoplasm. These acini may form small compact circular or tubular masses of cells, or they may form larger leaf shaped masses surrounding varying amounts of mucus. The acinar cells in these masses are larger than normal and contain large pale nuclei. In all the wall layers between the gland and the lumen there are many compact collections of a similar type

of cell, the shape and distribution of which indicate that these masses are remnants of gland ducts (Fig. 12).

The bronchial arteries have thickened walls involving chiefly the media and are not surrounded by increased fibrous tissue.

The nerves are not appreciably changed and are not surrounded by small round cells.

Comment.—The most significant pathologic findings are: (1) Partial occlusion of the lumina of bronchi and bronchioles by exudate. (2) Infiltration of the subepithelial layer of bronchi and bronchioles by small round cells and eosinophil cells. (3) Infiltration of mucous glands by small round cells. (4) Hyperplastic changes in acinar cells of bronchial mucous glands. (5) Slight hypertrophy of the right heart.

The etiology of the asthmatic attacks in this case is not clear. The history of attacks since early childhood with a free period following a change of climate and then a recurrence, could be interpreted either as sensitiveness to food or animal proteins or to infection in the respiratory tract.

Case 6.—*History.*—Infant M., female, aged 15 months. Normal delivery. Birth weight 8½ pounds. Family history is negative. Breast fed for three months with a gain in weight to 15 pounds, but because of a facial eczema she was put on artificial feedings by the family physician. At the eighth month she was admitted to the Children's Memorial Hospital Dispensary with eczema and weighing 11 pounds. Under dispensary management the skin condition improved and the weight increased. At the age of 12 months she was brought into the Children's Memorial Hospital suffering from a pharyngitis, accompanied by dyspnea and cyanosis of the face.

First Examination.—Well nourished. Temperature 101.6 F.; pulse, 160. Head: negative except for reddening of both ear drums, cyanosis of the lips and facial eczema. Neck, marked cervical adenopathy. Thorax: barrel shaped, markedly emphysematous and hyperresonant; marked expiratory stridor; squeaky râles all over the chest. Abdomen: distended and slightly tympanitic. Genitalia and extremities negative. Small doses of epinephrin and atropin gave only slight relief. The dyspnea and stridor gradually subsided, but the eczema persisted. Pus was obtained from both ears after paracentesis. Urticarial lesions appeared on the abdomen, back, groin and face one-half hour after a feeding of farina, and subsequent cutaneous protein sensitization tests revealed a marked degree of sensitiveness to wheat proteins and to whole wheat and a slight degree of sensitiveness to cow's milk and to lactalbumin. The reactions to orange, egg yolk, egg albumin, beef, mutton, navy bean, spinach, carrot, cocoa, coffee, rye, oats and barley were negative. Wheat protein and whole wheat, both of which gave definite reactions, were eliminated from the diet and oatmeal substituted as the cereal.

Course.—At the end of one week, the patient was discharged from the hospital with very little bronchitis, no râles in the chest, and much less of the facial skin involvement. Six days later she was again admitted to the hospital suffering from an asthmatic attack which may have been brought on by the ingestion of wheat containing food. Four days later the patient was again discharged from the hospital with very little bronchitis, no râles in the chest and no eczema. For two months her condition improved, wheat containing foods having been gradually added to the diet, then she was again admitted to the hospital suffering from a cold in the head and an attack of asthma.

Second Examination.—Leukocytes, 7,950. Hemoglobin (Sahli), 96 per cent. Respiration, 64. A fairly well-developed and nourished female child with

respiratory difficulty and markedly cyanotic. She has a marked respiratory wheeze and the accessory respiratory muscles are used. The head is inclined to be square shaped and is held in a retracted position. The anterior fontanel is open and bulging and quite tense. Craniotabes is present. Skin of the cheeks is rough. Chest is inclined to be barrel shaped, and on percussion there is a hyperresonant note over the entire chest. The heart tones are hard to hear on account of the wheezing noises in the chest. The liver is two fingers' breadth below the costal margin in the mammary line. The spleen is not palpable. There are no tumor masses in the abdomen. The knee reflexes are active. The use of epinephrin, atropin and benzyl benzoate afforded only transient relief and the patient died about thirty-two hours after entering the hospital. The postmortem examination was made a few hours later by Dr. W. G. Hibbs.

Necropsy Report: (Only the most important findings are given.)

Anatomic Diagnosis.—Huge hypertrophic emphysema of the lungs. "Barrel-shaped" chest. Downward displaced diaphragm and liver. Hypertrophy of the right ventricle of the heart. Slight passive hyperemia of the liver, kidneys and skin. Hypertrophy of the thymus, cervical and mesenteric lymph nodes, the spleen, the lymphoid tissue of the base of the tongue and the lymph nodes of the ileum. Narrowed aorta (congenital). Fatty changes of the liver. Needle puncture wounds of the upper arms. Suppurative vaginitis (gonococcus). Hyperemia of the uterus and adnexa. Eczema (?) of the skin of the face.

Macroscopic Examination.—This is the body of a white, female child about 15 months old. Only the front teeth, both lower and upper, are present. Body nourishment is fair. The chest is noticeably "barrel-shaped." The lower one third of the sternum is raised about 1 cm. above the general level of the corpus sterni as the body lies on the table. The abdominal wall in front is slightly distended and rigid. The external genitalia and anus are unchanged. The skin of the face is hard, crusted and in places scaled (eczema?). The content of cranium is not examined (permission refused). Abdomen: The uterus and its appendages are markedly hyperemic. The diaphragm on the right side is pushed down to opposite the level of the fifth rib, the fifth interspace on the left. The lower level of the liver is 1 cm. below the costal arch. The gallbladder is normally free. The urinary bladder is unchanged. The peritoneum is everywhere smooth and shiny. Thorax: The costal cartilages are soft. The lungs completely fill the pleural cavities. The lungs are free of adhesion. The pleura is everywhere smooth and shiny. There is no free fluid in the pleural sacs. The lungs and intact trachea from below the thyroid cartilage are removed en masse (weight 1677.5 gm.), and put into 10 per cent. liquor formaldehyd for later detailed study. Heart: the pericardial sac contains about 3 c.c. of clear fluid. The apex of the heart consists of right and left ventricles and in front the right ventricle is as broad as the left. The pulmonary artery contains only fluid blood. The pulmonary cusps are unchanged, as are the tricuspid leaflets. The right ventricle between base and apex is 1.5 cm. thick. There is no change of the linings of the carotid arteries, the inferior vena cava and the aorta. The aorta is about two thirds the usual size and measures 12 mm. in greatest diameter. Lymphoid tissue: The deep cervical lymph nodes are from 5 to 18 mm. in diameter. They are firm, pale yellow and generally hyperplastic. The tonsils and the lymph nodules of the base of the tongue are about twice the normal size. Kidneys: The kidneys together weigh 62 gm. The cortical striations are well defined and the cortices are uniformly from 3 to 5 mm. thick. They are grossly unchanged. Weights of organs: Suprarenals together, 5 gm.; thyroid, 2.5 gm.; thymus, 15 gm.; spleen, 20.2 gm.; pancreas, 7.5 gm.; liver, 293.5 gm.; heart, 57.5 gm.

Gross Description.—The lobes of both lungs are distended by air (Fig. 13), float high in water, and are soft and feathery to touch. The pleural surfaces

everywhere are smooth and shining and are grayish in color, except for a few scattered brownish slightly depressed, sharply defined, nodule-like areas measuring about 0.5 cm. in diameter. There are some large emphysematous bullae in the apices and in the anterior margins of both lungs. The trachea and the main bronchi contain a small amount of mucopurlent tenacious exudate and their epithelial lining is reddened. The peritracheal and peribronchial lymph glands are conspicuous and their cut surfaces are reddish gray in color. The cut surfaces of all lobes of both lungs are dry, grayish red in color and are air filled except for the above described nodule-like areas which are distributed from the hilum to the pleural surface. The connective tissue septae throughout the lungs are conspicuous. A few of the medium sized and smaller bronchi are patent but most are almost or completely occluded by a grayish tenacious exudate and their walls appear thickened. The larger blood vessels especially in the lower lobes of both lungs are distended by blood.

Fig. 13.—Case 6. Food asthma. Infant. Lungs showing acute emphysema.

Microscopic Description.—The structure of the different lobes of both lungs varies so little that a separate description of each is not necessary. The pleura throughout appears normal in structure and variations in thickness depend on the amount of air in the underlying tissue. Numerous connective tissue septa extending into the lung from the pleura are very conspicuous grossly and microscopically and sharply mark off definite units of lung tissue. These septa are composed of fibrous tissue which contains a few distended blood vessels and a few scattered lymphoid cells. There are also a few small collections of lymphoid cells beneath the pleura. The apices and anterior margins of both lungs are markedly emphysematous, the alveoli being widely distended and their walls thin and in places ruptured. The alveoli in the greater part of both lungs are air filled, moderately distended, and their walls of normal structure and thickness. The bronchioles and their finer divisions in these portions of the lungs are dilated and contain a more or less mucinous exudate in which there are varying numbers of polymorphonuclear neutrophil cells and

a few polymorphonuclear eosinophil cells. The epithelium is intact and is folded in only the contracted bronchioles. The basement membrane is not conspicuous. The subepithelial layer is relatively thin and contains only a few lymphoid cells and an occasional polymorphonuclear eosinophil cell. The muscle layer is scanty and irregular in distribution. The other connective tissue layer is thin and contains throughout numerous small definite lymphoid nodules with distinct germ centers.

The remainder of the lung tissue falls into two distinct groups which differ markedly from each other and from that already described. One of these is composed of definite areas from 0.5 to 1.5 cm. in diameter which are sharply marked off by septa and are scattered irregularly throughout both lungs. The alveoli are small and almost completely filled by a fine granular precipitate containing a few polymorphonuclear neutrophil cells and a few red blood cells. The walls of these alveoli are thickened by distended capillaries. The bronchioles and their divisions are occluded by a mucous exudate containing many polymorphonuclear neutrophil cells, a few polymorphonuclear eosinophil cells and a few epithelial cells. The epithelium of the bronchioles is slightly thickened and the ciliated border is fused with the mucus in the lumen. The subepithelial layer is thin and contains a few round cells. The muscle layer is scanty and irregular in distribution. The outer connective tissue layer is increased in amount and in many bronchioles is infiltrated by lymphoid cells and polynuclear eosinophil cells which in some are grouped about blood vessels. Distinct lymph nodules are prominent in the walls of some of the bronchioles.

The other group of well defined areas are more scattered, air free, somewhat smaller in size and retain, as the only evidence of lung tissue, small contracted bronchioles whose lumina are completely occluded by a mucous exudate containing a few polynuclear neutrophile cells absorption atelectasis (Plate 2, D). The major portion of these areas consist of closely crowded small mononuclear cells, among which are a few small capillaries, fibroblasts, large mononuclear cells (endothelial cells) and polymorphonuclear neutrophil and eosinophil cells. The blood vessels are small and are surrounded by an abnormal amount of fibrous tissue.

The small and medium sized bronchi in all parts of the lungs vary greatly in appearance, and these variations seem to bear no definite relation to the abnormal lung tissue. Approximately two thirds of these bronchi are almost, or completely occluded by a mucous exudate (Plate 3, A) which contains varying numbers of polymorphonuclear neutrophil and eosinophil cells, a few desquamated epithelial cells, but no fibrin and showing in longitudinal sections distinct layer formation. The epithelium is intact, thrown into shallow folds and contains a few polymorphonuclear eosinophil cells. The outer ciliated border seems to fuse with the content of the lumina. The basement membrane is not prominent. The subepithelial layer is thin and contains only a few lymphoid cells and a few polymorphonuclear eosinophil cells. The muscle layer is thin and irregularly distributed. The mucous glands are very prominent, their acini large and separated from each other by a loose connective tissue containing only a few lymphoid cells and a few polymorphonuclear eosinophil cells. Outside the muscle layer the collections of polymorphonuclear eosinophil cells (Plate 3, B and C) usually surrounding moderately distended blood vessels, make a striking picture. The cartilages are of normal appearance. The outer fibrous tissue layer is prominent. The blood vessels are distended only in both lower lobes. The peribronchial nerves and nerve sheaths are not infiltrated by cells and are unchanged. The lymph nodes along all bronchi are very numerous and prominent and contain large germ centers with scattered cells, and their sinuses contain a few polymorphonuclear eosinophil cells. The remainder of the small and medium sized bronchi are widely dilated and contain small amounts of mucous exudate similar to that described above. The walls are similar in structure, except that they are thinner. In many sections, occluded and distended bronchi lie

side by side. The main and large bronchi contain varying amounts of mucus. Their walls except for the normal structural differences, are similar to the walls of the medium sized bronchi. The collection of polymorphonuclear eosinophil cells about small blood vessels between the muscle layer and the cartilages is very striking throughout the whole bronchial tree. The peribronchial lymph glands are numerous and very large. Their sinuses are distended and the germ centers prominent and all glands contain many eosinophil cells. A slight anthracosis is present. The thyroid, parathyroid, liver, pancreas, suprarenals and kidneys are normal in structure. The sinuses of the spleen are filled with blood and the splenic pulp contains large numbers of polynuclear eosinophil cells. The cervical and mesenteric lymph glands are large, the germ centers are prominent and have loosely arranged cells but no evidence of necrosis, and some contain many distended blood vessels. All lymph glands examined, except the mesenteric, contain many polymorphonuclear eosinophil cells. The thymus is well developed and the cortical portions contain many diffusely scattered, irregularly shaped, deeply stained cellular masses, Hassall's corpuscles. The cortical and medullary portions contain many groups of mononuclear eosinophil cells (Plate 3, D).

Comment.—The chief pathologic findings in this infant may be summarized as follows: (1) complete occlusion of the majority of the middle sized and smaller bronchi by mucus. (2) Two distinct stages in the formation of areas which are interpreted as evidences of past asthmatic attacks (absorption atelectasis). (3) Extreme eosinophilia of the following structures: Bronchial wall, mucous content of bronchial lumen, mucous glands, spleen, lymphoid tissues and thymus. (4) Thickening of the walls of the bronchi and blood vessels. (5) Cell inclusions in the thymus. (6) Hypertrophy of entire lymphoid apparatus. (7) Narrowing of aortic arch. (8) Enlargement of the thymus.

The last three statements point to the possibility of this case being one of status lymphaticus, although the characteristic areas of necrosis in the lymph glands, which have been emphasized,[23] are absent. The clinical picture also is not characteristic.

The history of head colds and bronchitis preceding severe attacks is a point of interest. The history of eczema, of positive cutaneous tests for wheat proteins, of improvement in condition following the use of a wheat free diet and the lack of microscopic evidence of former infections in the subepithelial layer and in the mucous glands place this case in the category of the food asthmas.

MEASUREMENTS OF BRONCHI AND BRONCHIOLI

Since the abnormal inflation of the lungs and the impairment of respiration in asthma are undoubtedly associated with a narrowing of the lumina of the bronchial tubes, it is in order to ask what factors are involved in the production of this stenosis. Naturally, the attention is directed toward the smooth muscle fiber system, which acts by

23. Symmers, D.: Status Lymphaticus, Am. J. M. Sc. **156**:40, 1919.

diminishing the size of the tube, and toward the exudative system (i. e., *a*, mucous epithelium; *b*, glands, and *c*, blood vessels and capillaries) which adds to the stenosis by its increase in size and in secretory activity. Both these systems, of course, are regulated in function by the nervous system.

If the bronchial muscle contracts, certain temporary changes in the structure of the walls must immediately occur, i. e., there must be an increase in the thickness of the muscle bundles and in the height of the epithelial folds. The question arises then whether repeated spasmodic attacks over a long period of time might not ultimately cause a true hypertrophy of the smooth muscle system as well as changes in other portions of the tubes. Previous workers noted that their cases seemed to show an increase in the thickness of the bronchial wall

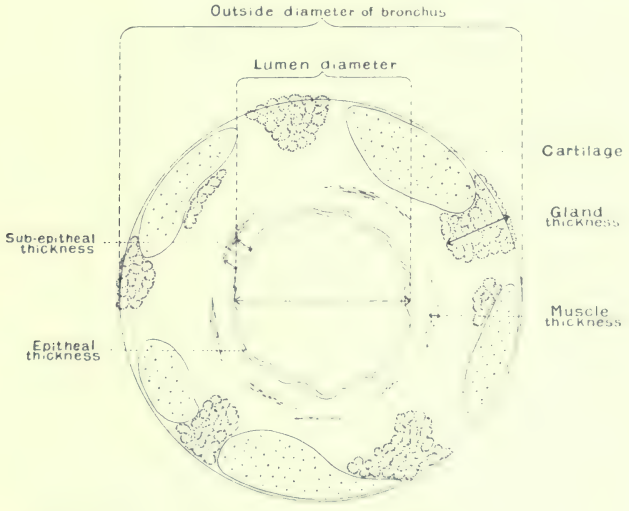

Fig. 14.—Diagrammatic view of the cross section of a bronchus to show the method used in making the measurements of the different structures.

but to our knowledge no actual proof has yet been given for the existence of such a change. In order to obtain definite evidence on this important point, we have made a comparison of the measurements of the bronchial structures of asthmatic and nonasthmatic individuals.

The typical transverse sections of the bronchi and bronchioli on which histologic studies were made were measured microscopically with the micrometer, in the following manner: a = mean distance between outer borders, b = mean diameter of lumen, c = epithelial layer thickness, d = basement membrance thickness, e = subepithelial layer thickness, f = muscle bundle thickness, and g = mucous gland diameter (Fig. 14). The classification of bronchi as to size is based on the measurement between outer borders and not on the diameter of the

lumen, as is usually done. This method gives, we believe, especially in all cartilage-bearing tubes, a better basis for comparison than the usual method. The measurement of the lumen takes into account the slight irregularities due to the shallow longitudinal epithelial folds. The epithelial thickness is measured from the attachment of the cilia to the point of contact with the basement membrane. The basement membrane thickness is measured only in those sections in which its borders are

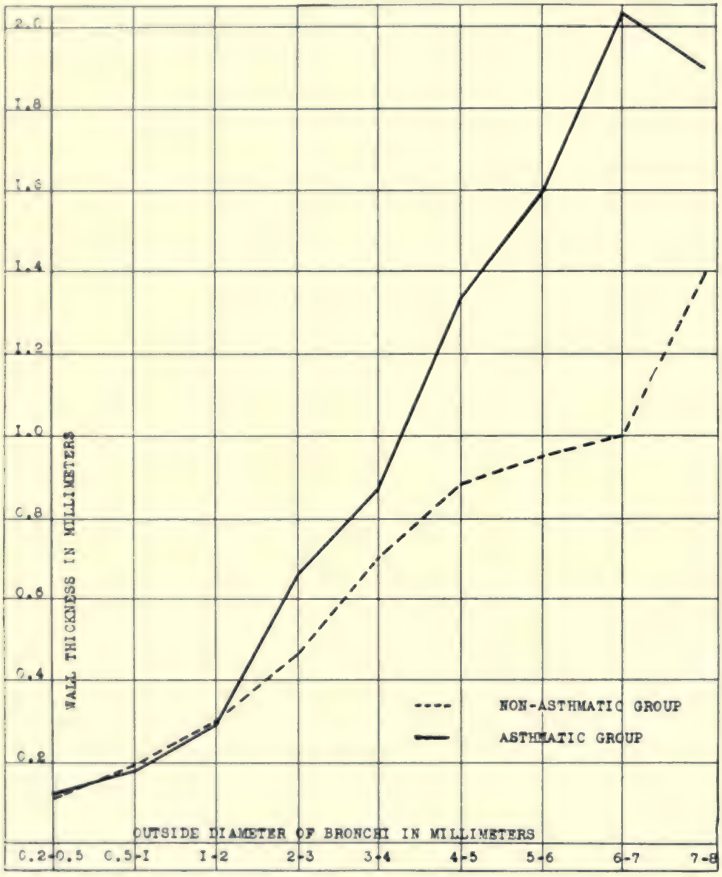

Fig. 15.—Graphs showing comparison of wall thickness.

distinct. The measurement of the subepithelial layer, which includes all tissues between the basement membrane and the muscle layer, also takes into account the longitudinal epithelial folds. The diameters of the muscle bundles and of the mucous glands are measured at right angles to the tube lumen. All measurements are made, as far as possible, in the tube segment with most uniform structures, otherwise the mean of several measurements is taken.

Thirteen individuals, six asthmatic and seven nonasthmatic constitute our material on which the measurements of the bronchial structures were made. Some important data concerning them are given in Table 1.

TABLE 1.—Data Regarding Individuals Whose Bronchi Were Measured

	Sex	Age	Death Due to	Chief Complaint
1. B.	F	55 yrs.	Suicide—drowning	Asthma
2. G.	M	55 yrs.	Asthma	Asthma
3. Le.	M	29 yrs.	Abscess of lung	Asthma
4. E. L.	M	29(?) yrs.	Acute fatal anaphylaxis	Asthma—horse
5. L.	F	17 yrs.	Acute streptococcus infection	Asthma
6. Baby A.	F	15 mos.	Asthma	Asthma
7. Gr.	M	70 yrs.	Cardiac decompensation	Chronic bronchitis
8. Br.	F	62 yrs.	Carcinoma of stomach	Carcinoma
9. A.	M	50 yrs.	Pneumonia of l. lower lobe	Chronic bronchitis
10. N. L.	M	27 yrs.	Suicide, jugulars severed	Psychosis
11. R.	M	26 yrs.	Suicide, shot through heart	Psychosis
12. B.	M	25 yrs.	Brain abscess	Brain abscess
13. Baby N.	F	15 mos.	Enteritis	Enteritis

Numbers 7 and 9 are omitted from all graphs because of the questioned diagnosis of early asthma. Numbers 6 and 13 are also omitted from all graphs because of the youth of these patients, however, separate graphs, not reproduced here, show similar increased thickness in the tubes of the asthmatic child.

The measurements obtained from these subjects are given in Tables 2 and 3.

There are certain unavoidable apparent sources of error in the figures given in Tables 2 and 3 and in order to offset these the largest number of measurements possible are taken. Groupings by tube size is open to the objection that more measurements may be made near one extreme in one group than in the other, but our figures show that such occurrences influence the graphs about equally. Because of the marked variations in the size of the bronchi at the same distance from the bifurcation of the trachea in different persons, the criticism that the increase in wall-thickness in our asthmatic subjects may be due to a uniform contraction of all tubes cannot be answered definitely. The size and thickness of the bronchial cartilaginous plaques vary greatly, depending somewhat on nearness to a bifurcation, so that measurement of these plaques is of little value in determining location. However, the longitudinal folding of the epithelial layer, which may be one evidence of contraction, shows no consistent variation in the two groups of cases. It will be noted that tubes of the same size in the same individual show marked variations in structures but these are as striking in one group as in the other. Differences in numbers of measurements obtainable from different persons are unavoidable—the greatest numbers being obtained in those whose lungs furnished the greatest number of typical sections. The number of typical sections obtainable from each individual is limited by the amount of lung tissue originally obtained and the amount of tissue uninfluenced by other pathologic conditions such as pneumonia, etc.

TABLE 2.—Measurements in Millimeters of Bronchi and Bronchioli. Adults

These tables are arranged so that the outside diameter of the bronchial tubes is taken as the fixed standard of comparison. The differences in the size of structures of the asthmatic and non-asthmatic individuals as obtained from these measurements are demonstrated graphically in Figures 15, 16 and 17.

Name	Diagnosis	Outside Diameter	Inside Diameter	Wall Thickness	Epithelial Thickness	Basement Membrane Thickness	Subepithelial Thickness	Muscle Thickness	Gland Diameter
Normal L.	Normal	0.22	0.12	0.05	0.013	.....	0.016	0.010	....
Normal B.	Brain abscess.	0.28	0.18	0.05	0.016	.....	0.013	0.010	....
Mr. E. L.	Asthma	0.28	0.18	0.05	0.025	.....	0.010	0.016	....
Miss L.	Asthma	0.30	....	....	0.019	.....	0.016	0.013	....
Mr. G.	Asthma	0.32	0.14	0.09	0.016	.....	0.036	0.026	....
Mr. G.	Asthma	0.33	0.17	0.08	0.016	.....	0.016	0.023	....
Miss L.	Asthma	0.35	....	....	0.022	.....	0.016	0.012	....
Normal L.	Normal	0.36	0.14	0.11	0.010	.....	0.007	0.010	....
Normal L.	Normal	0.36	0.12	0.12	0.016	.....	0.033	0.020	....
Mr. E. L.	Asthma	0.36	0.12	0.12	0.039	.....	0.028	0.028	....
Mr. E. L.	Asthma	0.38	0.14	0.12	0.023	.....	0.028	0.021	....
Normal B.	Brain abscess.	0.40	0.15	0.125	0.016	.....	0.016	0.026	....
Normal B.	Brain abscess.	0.42	0.10	0.16	0.016	.....	0.016	0.010	....
Mr. R.	.....	0.44	0.28	0.08	0.013	.....	0.013	0.013	....
Mr. G.	Asthma	0.48	0.15	0.165	0.020	.....	0.033	0.050	....
Normal B.	Carcinoma	0.50	0.21	0.145	0.013	.....	0.019	0.013	....
Normal B.	Brain abscess.	0.50	0.14	0.18	0.013	.....	0.010	0.033	....
Mr. E. L.	Asthma	0.50	0.14	0.18	0.036	.....	0.023	0.050	....
Mr. E. L.	Asthma	0.50	0.26	0.12	0.033	.....	0.023	0.019	....
Mr. Gr.	Bronchitis	0.50	0.22	0.14	0.013	.....	0.020	0.033	....
Normal B.	Carcinoma	0.58	0.25	0.165	0.016	.....	0.026	0.033	....
Normal L.	Normal	0.59	0.30	0.145	0.029	.....	0.033	0.018	....
Mr. R.	.....	0.61	0.24	0.185	0.013	.....	0.026	0.023	....
Miss L.	Asthma	0.64	0.10	0.27	0.033	.....	0.023	0.019	....
Mr. R.	.....	0.64	0.22	0.21	....	.....	0.023	0.019	....
Mr. R.	.....	0.67	0.24	0.215	0.033	.....	0.026	0.026	....
Normal L.	Normal	0.68	0.30	0.19	0.025	.....	0.042	0.025	....
Mr. R.	.....	0.70	0.24	0.23	0.033	.....	0.033	0.037	....
Mr. Le.	Asthma	0.70	0.20	0.25	.....	.....	0.042	0.016	....
Mr. R.	.....	0.71	0.30	0.205	0.021	.....	0.023	0.026	....
Mr. R.	.....	0.71	0.26	0.225	0.033	.....	0.050	0.049	....
Mr. R.	.....	0.71	0.31	0.20	0.013	.....	0.033	0.033	....
Mrs. B.	Asthma	0.71	0.40	0.155	.....	0.0066	0.028	0.014	....
Normal B.	Brain abscess.	0.80	0.52	0.14	0.018	.....	0.021	0.021	....
Normal L.	Normal	0.80	0.60	0.10	0.019	.....	0.016	0.019	....
Mrs. B.	Asthma	0.86	0.60	0.13	.....	0.0066	0.014	0.014	....
Normal L.	Normal	0.87	0.70	0.085	.....	.....	0.016	0.016	....
Normal L.	Normal	0.87	0.48	0.195	0.016	.....	0.026	0.019	....
Mr. R.	.....	0.87	0.32	0.275	0.019	.....	0.050	0.039	....
Mr. Gr.	Bronchitis	0.88	0.66	0.11	.....	.....	0.056	0.042	....
Normal L.	Normal	0.91	0.40	0.255	0.052	.....	0.042	0.042	....
Mr. G.	Asthma	1.00	0.62	0.19	0.040	.....	0.026	0.033	....
Mrs. B.	Asthma	1.00	0.70	0.15	.....	0.010	0.070	0.100	....
Mrs. B.	Asthma	1.00	0.70	0.15	.....	0.0066	0.028	0.014	....
Mr. R.	.....	1.00	0.53	0.235	0.013	.....	0.066	0.039	....
Mr. R.	.....	1.10	0.50	0.30	0.033	.....	0.033	0.040	....
Normal B.	Brain abscess.	1.10	0.64	0.23	.....	.....	0.026	0.016	....
Mr. G.	Asthma	1.12	0.62	0.25	0.026	.....	0.033	0.026	....
Normal B.	Brain abscess.	1.20	0.80	0.20	0.013	.....	0.084	0.028	....
Normal B.	Brain abscess.	1.20	0.80	0.20	.....	.....	0.070	0.028	....
Mr. R.	.....	1.28	0.63	0.325	0.023	.....	0.083	0.050	....
Normal L.	Normal	1.40	0.86	0.270	0.025	.....	0.070	0.019	....
Mr. G.	Asthma	1.43	0.60	0.415	0.033	0.0066	0.040	0.037	0.17
Mr. G.	Asthma	1.60	1.16	0.22	0.045	.....	0.056	0.033	0.18
Normal Br.	Carcinoma	1.68	1.00	0.34	0.023	.....	0.039	0.050	....
Miss L.	Asthma	1.80	1.10	0.35	0.050	.....	0.050	0.033	....
Normal Br.	Carcinoma	1.80	1.16	0.32	.....	.....	0.033	0.033	....
Mr. A.	Bronchitis	1.80	0.92	0.44	.....	.....	0.056	0.140	0.50
Normal Br.	Carcinoma	1.84	1.16	0.34	.....	.....	0.033	0.033	....
Mrs. B.	Asthma	1.90	1.50	0.20	.....	0.010	0.015	0.043	....
Normal L.	Normal	1.95	1.25	0.35	.....	.....	0.056	0.040	0.46
Normal Br.	Carcinoma	1.96	1.27	0.345	.....	.....	0.033	0.033	....
Mrs. B.	Asthma	2.10	0.62	0.74	.....	0.010	0.090	0.110	0.31
Mr. E. L.	Asthma	2.10	0.92	0.59	0.060	.....	0.068	0.150	....
Mrs. B.	Asthma	2.20	1.30	0.45	0.033	0.012	0.042	0.042	0.30
Mr. R.	.....	2.20	1.60	0.30	.....	.....	0.053	0.026	....
Normal Br.	Carcinoma	2.20	1.00	0.60	0.045	0.0066	0.070	0.070	0.24
Mr. G.	Asthma	2.28	1.16	0.56	.....	.....	0.070	0.084	....
Mr. A.	Bronchitis	2.30	1.10	0.60	.....	0.0099	0.100	0.140	0.50
Mr. A.	Bronchitis	2.40	1.00	0.70	.....	.....	0.120	0.180	0.28
Mrs. B.	Asthma	2.40	1.00	0.70	.....	.....	0.100	0.100	0.58
Mr. G.	Asthma	2.40	1.00	0.70	0.065	.....	0.056	0.056	0.50

TABLE 2.—Measurements in Millimeters of Bronchi Bronchioli. Adults—(*Continued*)

Name	Diagnosis	Outside Diameter	Inside Diameter	Wall Thickness	Epithelial Thickness	Basement Membrane Thickness	Subepithelial Thickness	Muscle Thickness	Gland Diameter
Mr. E. L.	Asthma	2.40	1.10	0.65	0.006	0.0072	0.056	0.056	0.26
Miss L.	Asthma	2.50	1.40	0.55	0.084	0.0066	0.026	0.050	0.29
Mrs. B.	Asthma	2.60	2.20	0.20	....	0.010	0.043	0.043	....
Normal L.	Normal	2.70	1.70	0.50	0.033	.....	0.052	0.026	....
Miss L.	Asthma	2.70	1.60	0.55	0.084	0.0066	0.026	0.056	0.36
Mr. Le.	Asthma	2.80	1.20	0.80	.....	.....	0.140	0.126	....
Mrs. B.	Asthma	2.90	1.80	0.55	.....	0.012	0.140	0.086	0.32
Mrs. B.	Asthma	3.00	1.50	0.75	.....	.....	0.070	0.070	0.88
Mr. A.	Bronchitis	3.00	1.46	0.77	.....	0.0099	0.070	0.140	0.72
Mr. Le.	Asthma	3.00	0.70	1.15	.....	.....	0.140	0.180	0.50
Mr. Le.	Asthma	3.00	0.70	1.15	.....	.....	0.180	0.200	0.36
Mr. E. L.	Asthma	3.00	1.70	0.65	0.083	0.0072	0.100	0.110	0.37
Mrs. B.	Asthma	3.12	1.72	0.70	.....	.....	0.100	0.180	0.86
Mrs. B.	Asthma	3.20	1.40	0.90	.....	0.010	0.070	0.086	0.48
Miss L.	Asthma	3.20	1.84	0.68	0.058	0.0072	0.054	0.066	0.28
Mr. Gr.	Bronchitis	3.33	1.68	0.825	.....	.....	0.070	0.084	0.34
Mr. Gr.	Bronchitis	3.33	1.74	0.795	.....	.....	0.045	0.039	0.29
Mr. G.	Asthma	3.40	1.80	0.80	.....	.....	0.056	0.070	....
Mr. L.	Asthma	3.40	1.30	1.05	0.078	.....	0.083	0.110	0.40
Mrs. B.	Asthma	3.42	1.56	0.93	.....	.....	0.110	0.160	0.86
Mr. A.	Bronchitis	3.50	1.40	1.05	0.056	.....	0.085	0.220	0.14
Normal Br.	Carcinoma	3.50	1.75	0.875	0.050	0.0066	0.040	0.070	0.38
Mr. G.	Asthma	3.50	1.94	0.78	0.056	.....	0.070	0.140	0.34
Mrs. B.	Asthma	3.60	1.80	0.90	.....	0.010	0.100	0.100	0.90
Normal L.	Normal	3.80	1.75	1.025	0.045	.....	0.100	0.095	0.30
Mr. R.	.....	3.80	2.60	0.60	0.050	.....	0.084	0.120	0.20
Mrs. B.	Asthma	4.00	1.90	1.05	.....	0.010	0.100	0.180	0.70
Normal L.	Normal	4.00	3.00	0.50	0.033	.....	0.100	0.042	0.30
Normal L.	Normal	4.00	3.00	0.50	0.039	.....	0.033	0.060	0.32
Normal L.	Normal	4.20	2.28	0.96	.....	.....	0.110	0.084	0.44
Mrs. B.	Asthma	4.20	1.80	1.20	.....	0.010	0.170	0.200	0.70
Mr. Gr.	Bronchitis	4.50	....	....	0.056	.....	0.120	0.120	0.80
Mr. Gr.	Bronchitis	4.50	2.40	1.05	0.050	0.0066	0.090	0.180	0.60
Mrs. B.	Asthma	4.64	2.80	0.92	.....	.....	0.100	0.140	0.86
Mrs. B.	Asthma	4.80	2.24	1.28	.....	.....	0.100	0.130	0.58
Mrs. B.	Asthma	4.80	0.86	1.97	.....	.....	0.220	0.200	1.10
Normal B.	Brain abscess	5.00	3.50	0.75	.....	.....	0.086	0.090	0.30
Normal Br.	Carcinoma	5.04	3.14	0.95	0.042	.....	0.066	0.072	0.70
Miss L.	Asthma	5.06	2.50	1.28	0.063	0.0066	0.100	0.140	0.72
Normal L.	Normal	5.10	3.20	0.95	0.056	.....	0.115	0.084	0.32
Mrs. B.	Asthma	5.44	1.92	1.96	.....	.....	0.260	0.260	1.00
Mr. Gr.	Bronchitis	5.60	2.54	1.53	.....	.....	0.200	0.200	0.71
Mrs. B.	Asthma	5.76	2.40	1.68	.....	.....	0.220	0.180	1.00
Mr. Gr.	Bronchitis	6.00	3.50	1.25	....	0.0066	0.120	0.190	1.00
Mr. G.	Asthma	6.08	4.08	1.00	0.071	0.0099	0.120	0.230	0.72
Mr. Gr.	Bronchitis	6.40	3.30	1.55	0.062	.....	0.056	0.110	0.62
Normal L.	Normal	6.50	5.00	0.75	0.050	.....	0.100	0.140	0.38
Normal L.	Normal	6.50	4.00	1.25	0.036	.....	0.115	0.056	0.18
Mrs. B.	Asthma	6.50	2.70	1.95	0.052	0.013	0.110	0.110	1.10
Mrs. B.	Asthma	7.00	2.52	2.24	.....	0.010	0.210	0.180	1.10
Miss L.	Asthma	7.15	3.95	1.60	0.056	0.0066	0.130	0.220	0.33
Mr. G.	Asthma	7.30	4.92	1.19	0.050	.....	0.157	0.140	0.60
Miss L.	Asthma	7.50	3.00	2.25	0.056	0.0066	0.140	0.240	1.00
Mr. Gr.	Bronchitis	7.50	4.20	1.65	0.056	0.0066	0.070	0.180	0.71
Normal B.	Brain abscess	8.00	5.50	1.25	.....	.....	0.112	0.126	0.58
Normal Br.	Carcinoma	8.00	5.00	1.50	0.050	0.0066	0.110	0.090	0.30
Mr. R.	.....	8.40	5.20	1.60	0.056	0.0066	0.042	0.120	0.50
Mr. R.	.....	8.40	5.72	1.34	0.056	.....	0.070	0.120	0.58
Normal B.	Brain abscess	8.50	5.50	1.50	0.056	0.0080	0.070	0.084	0.74
Mrs. B.	Asthma	8.50	5.00	1.75	0.033	0.010	0.200	0.200	1.30
Mr. G.	Asthma	8.50	5.00	1.75	0.050	0.011	0.10	0.160	0.33
Mr. Gr.	Bronchitis	9.20	5.20	2.50	.....	0.0066	0.12	0.140	0.88
Mr. G.	Asthma	9.00	4.50	2.25	0.071	0.0099	0.120	0.090	0.72
Normal B.	Brain abscess	9.50	5.50	2.00	.....	0.0066	0.084	0.070	0.58
Mr. A.	Bronchitis	10.00	6.50	1.75	.....	.....	0.200	0.190	0.88
Mr. G.	Asthma	10.00	6.00	2.00	0.050	0.0099	0.120	0.240	0.31
Normal L.	Normal	11.00	6.50	2.25	0.056	0.0066	0.120	0.256	0.80
Mr. Gr.	Bronchitis	11.00	6.50	2.25	0.056	0.0080	0.070	0.180	0.71
Mr. Gr.	Bronchitis	12.00	6.00	3.00	.....	.....	0.200	0.250	0.88
Mr. A.	Bronchitis	12.00	7.00	2.50	.....	0.0066	0.200	0.350	0.88
Mr. Gr.	Bronchitis	12.00	6.50	2.75	0.084	0.0099	.....	0.140	1.20
Miss L.	Asthma	12.00	8.50	1.75	0.071	0.0066	0.022	0.240	0.72

TABLE 3.—Measurements in Millimeters of Bronchi and Bronchioli. Infants

Name	Diagnosis	Outside Diameter	Inside Diameter	Wall Thickness	Epithelial Thickness	Basement Membrane Thickness	Subepithelial Thickness	Muscle Thickness	Gland Diameter
Infant A.....	Asthma.....	0.38	0.18	0.10	0.016	.....	.....	0.007	....
Infant N.....	Enteritis.....	0.38	0.20	0.09	0.013	.....	0.007	0.010	....
Infant N.....	Enteritis.....	0.50	0.38	0.06	0.016	.....	0.013	0.010	....
Infant A.....	Asthma.....	0.66	0.36	0.15	0.033	.....	0.070	0.056	....
Infant N.....	Enteritis.....	0.72	0.46	0.13	0.026	.....	0.016	0.013	....
Infant A.....	Asthma.....	0.92	0.62	0.15	.....	.....	0.019	0.010	....
Infant N.....	Enteritis.....	0.94	0.50	0.22	0.040	.....	0.020	0.017	....
Infant A.....	Asthma.....	1.00	0.46	0.27	.....	.....	0.016	0.016	....
Infant N.....	Enteritis.....	1.18	0.86	0.18	0.050	.....	0.033	0.017	....
Infant A.....	Asthma.....	1.40	1.00	0.20	.....	.....	0.028	0.042	....
Infant A.....	Asthma.....	1.40	0.50	0.45	0.036	.....	0.033	0.016	....
Infant N.....	Enteritis.....	1.54	0.86	0.34	0.050	.....	0.033	0.017	....
Infant A.....	Asthma.....	1.60	0.88	0.36	.....	.....	0.033	0.020	0.20
Infant N.....	Enteritis.....	1.80	1.00	0.40	0.050	.....	0.025	0.025	....
Infant N.....	Enetritis.....	1.80	1.00	0.40	0.050	.....	0.027	0.020	....
Infant A.....	Asthma.....	2.20	1.20	0.50	.....	.....	0.066	0.033	0.20
Infant A.....	Asthma.....	2.20	1.00	0.60	0.040	.....	0.070	0.024	0.36
Infant A.....	Asthma.....	2.20	1.40	0.40	.....	.....	0.070	0.042	....
Infant A.....	Asthma.....	2.40	1.10	0.65	.....	.....	0.084	0.042	0.22
Infant A.....	Asthma.....	2.40	1.60	0.40	0.039	.....	0.10	0.023	0.20
Infant N.....	Enteritis.....	2.40	1.56	0.42	0.042	.....	0.042	0.023	0.14
Infant N.....	Enteritis.....	2.56	1.56	0.50	0.042	.....	0.056	0.042	0.28
Infant A.....	Asthma.....	2.60	1.3	0.65	0.033	.....	0.084	0.042	0.42
Infant A.....	Asthma.....	2.64	1.36	0.64	0.033	.....	0.084	0.019	0.36
Infant N.....	Enteritis.....	2.68	1.54	0.57	0.040	.....	0.013	0.013	0.28
Infant A.....	Asthma.....	3.00	1.8	0.60	.....	.....	0.046	0.026	0.42
Infant A.....	Asthma.....	3.40	1.4	1.00	0.029	0.066	0.157	0.042	0.30
Infant A.....	Asthma.....	4.50	2.4	1.05	0.026	.....	0.140	0.10	0.70
Infant N.....	Enteritis.....	5.50	3.5	1.00	0.013	0.0066	0.084	0.146	0.30
Infant A.....	Asthma.....	5.60	2.40	1.60	0.033	0.0066	0.142	0.10	0.72
Infant A.....	Asthma.....	6.50	2.50	2.00	0.046	0.0066	0.10	0.056	0.42
Infant A.....	Asthma.....	7.50	3.50	2.00	0.042	.....	0.070	0.084	0.58
Infant N.....	Enteritis.....	7.50	4.50	1.50	0.036	.....	0.070	0.120	0.58

Figure 15 shows graphically the differences in wall thickness in the asthmatic and nonasthmatic subjects. Figures 16 and 17 show graphically the differences in the subepithelial layer and the muscle bundle thickness in the same groups of subjects. The increase in the wall thickness in asthmatics is accounted for only in part by the increased size of the subepithelial layer and of the muscle bundles. Measurements show, however, that the mucous glands in most of our asthmatic subjects are considerably enlarged, and as this gland system is present inside, outside and between the edges of the cartilaginous plaques, no adequate system of measurement could be devised to determine the actual increase due to gland changes. We recognize also the change produced by hyperemia in the tissues between the cartilages and muscle bundles, but this change is difficult to determine by measurements because of the influence of glandular changes. The tubes with an outside diameter of less than 2 mm. show in the graphs only a slight variation in thickness; the asthmatic group showing somewhat smaller measurements probably due to the influence of emphysema. The thickness of epithelium, subepithelial layer and muscle bundles in

divisions of the tubes below the bronchioli are practically the same in the two groups.

A great deal of caution must be observed in the interpretation of epithelial fold formation as evidence of abnormal bronchial contraction, as deep folds are frequently found postmortem in apparently normal bronchi and bronchioli much like the folding of the intima in arteries.

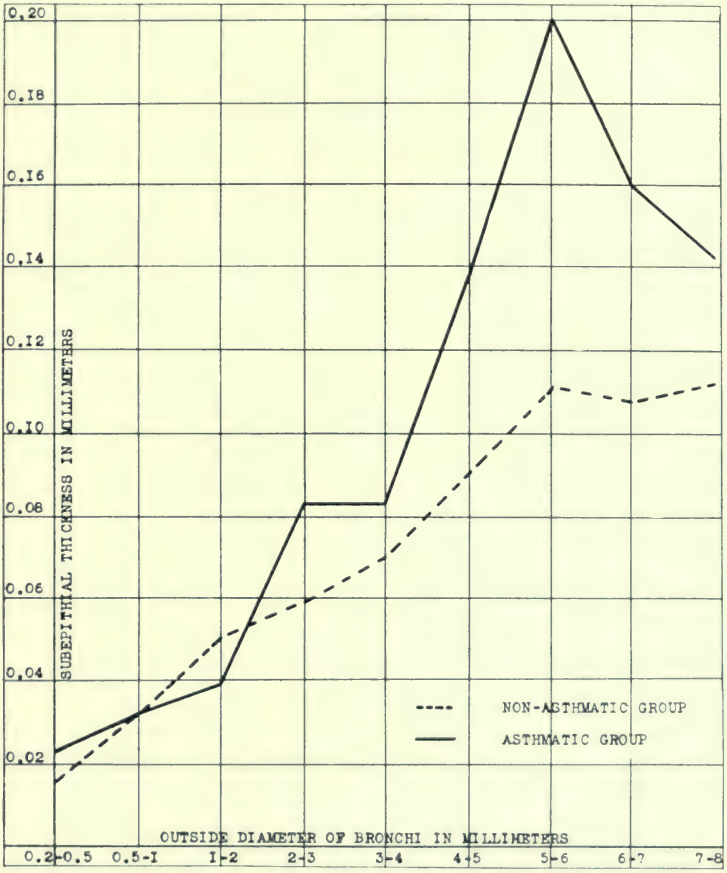

Fig. 16. Graphs showing comparison of subepithelial thickness.

We have examined the bronchi and bronchioli of many persons who died of various disorders and have gained the impression that the epithelial folding, when present in asthmatics, is of a greater degree than that found in nonasthmatic (Cases 2 and 4). The amount of variation is of about the same degree as that found in the normal and in the anaphylactic guinea-pig lung. Pictures like those shown in Figures 18 and 19 are not usually seen in the lungs of normal guinea-pigs when the animal is killed by a stroke on the head. It is just such

pictures, in addition to the theraputic (inhibitive) effect of epinephrin and atropin, which form the chief evidence for the bronchospastic origin of the acute emphysema of the anaphylactic shock in the guinea-pig. While asthma is a chronic condition and is frequently accompanied by varying degrees of emphysema, the acute paroxysmal attack is the

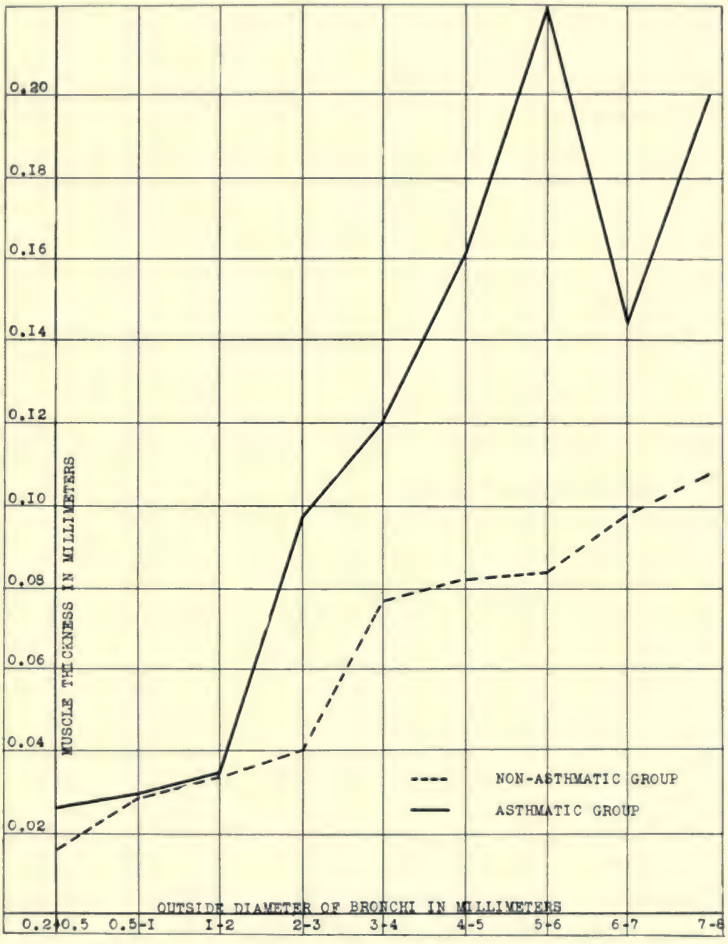

Fig. 17.—Graphs showing comparison of muscle thickness.

main characteristic symptom of the disease, and when associated with an acute stage of emphysema it forms the equivalent to the anaphylactic shock. In three of our cases (Cases 2, 3 and 4) the lumina of many small bronchi and bronchioli are almost occluded by folded epithelium (Plate 2, A and B; Fig. 9). The marked folding of the epithelium in our other cases is present but is not so striking as in the three cases cited.

TABLE 4.—General Summary of Clinical Data of All Cases.

Case	Reported by	Date	Age	Sex *	Occupation	Duration	Heredity	Associated Disorders	Cause of Death
1	v. Leyden	1886	40	♀	.....	From childhood	Not reported	.....	Died during an attack
2	Berkart	1889	37	♀	.....	14 yrs.	Not reported	.....	Died during an attack
3	E. Schmidt	1892	49	♀	Waitress	2-3 wks.	Not reported	Carcinoma in mediastinum; articular rheumatism	Died during an attack
4	A. Fraenkel	1898	63	♂	Carpenter	9-10 mos.	Not reported	Rheumatism; gout; bronchial catarrh (3 yrs.)	Died during an attack
5	A. Fraenkel	1900	48	♂	Not reported	At least 20 mos.	Not reported	.....	Died during an attack
6	Jezierski	1905	63	♂	Gardener	Probably about 4 yrs.	Father had asthma	Pneumonia	Pneumonia
7	Jezierski	1905	46	♀	Silk-weaver	12 yrs.	Not reported	.....	Died during an attack
8	Ellis	1908	27	♂	Coachman	At least 1 yr.	Not reported	Tachycardia	Died during an attack
9	Mönckeberg	1909	29	♂	Mason	3 yrs.	Not reported	Pneumonia at 12 and 18; psoriasis; anasarca	Died during an attack
10	H. Heizer	1911	2	..	.....	After 9th month	Negative	Eczema	Died during an attack
11	Tichmeneff	1913	29	♀	College student	From childhood	Not reported	Croup; nasal catarrh	Duodenal hemorrhage
12	Marchand	1915	53	♀	.....	23 yrs.	Mother had asthma and bronchitis	Bronchitis since 17th year	Died during an attack
13	Marchand	1915	45	♀	.....	At least 1 yr.	Not reported	.....	Died during an attack
14	Marchand	1918	48	♂	Chemical worker	2 yrs.	Aunt had asthma	.....	Died during an attack
15	Kamchorn and Ellis	1921	52	♂	Actor and fishmonger	From childhood	Father, father's mother, 1 uncle, 1 aunt and 1 child had asthma	.....	Heart failure
16	Huber and Koessler	1922	55	♀	Musician, housewife	5 yrs.	Negative	Bronchitis and coryza	Suicide by drowning
17	Huber and Koessler	1922	55	♂	Real estate	6 yrs.	Mother, 2 sisters, 1 brother and 1 child had asthma	Bronchitis	Died during an attack
18	Huber and Koessler	1922	17	♀	.....	From childhood	Negative	.....	Pericarditis and peritonitis
19	Huber and Koessler	1922	29	♂	Soldier	19 yrs.	Mother had asthma	Head colds	Fatal anaphylaxis
20	Huber and Koessler	1922	32	♂	Car-repairer	From childhood	Negative	.....	Abscess of lung; pneumonia
21	Huber and Koessler	1922	15 mo.	♀	.....	5 mos.	Negative	Eczema; undernourished	Died during an attack

* In this column, ♂ indicates male, and ♀ female.

The increased thickness of the muscle layer of the bronchi in all of the cases reported is apparent from our measurements. The muscular tissue is well developed, not only in the middle sized and small bronchi but even in the bronchioli. This increase in the size of the smooth muscles surrounding the bronchi may be due to a true increase in the size and quantity of the muscle cells, therefore a true hypertrophy, or it may be due to an increased contraction of the muscle. Increased contraction of a smooth muscle implies with few exceptions [24] increased muscular tonus and the increased thickness of a smooth muscle due

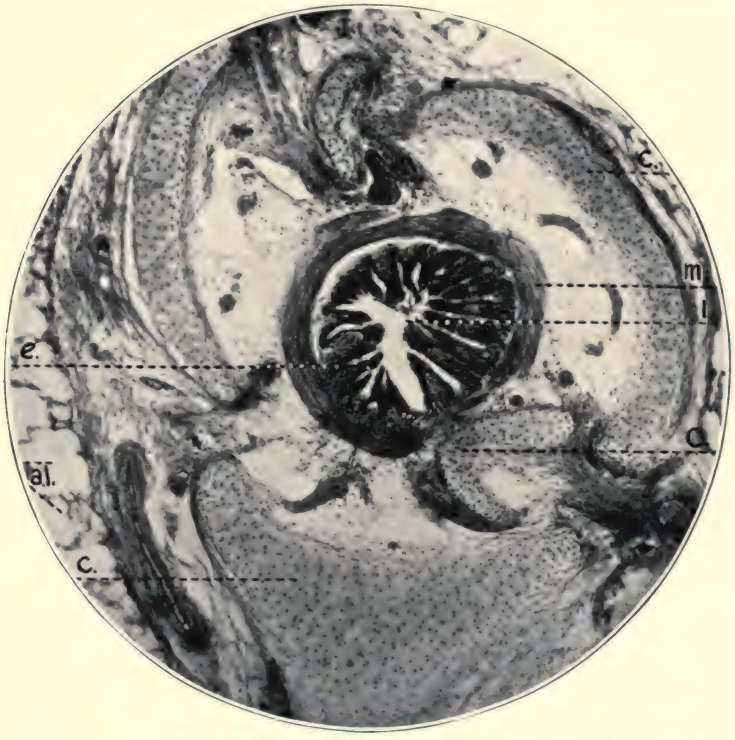

Fig. 18.—Guinea-pig. Fatal horse serum anaphylaxis. Large bronchus. Cross section. Epithelium deeply folded and almost obliterating the lumen. Muscle layer forms a distinct layer. There are no glands. *l* = lumen; *e* = epithelium; *m* = muscle; *c* = cartilage; *al* = alveoli. Magnification, 70 diameters.

to contraction is the morphological expression of hypertonus. These factors—hypertonus, repeated contraction, and hypertrophy are closely and functionally related and probably all three are involved in the production of the increased thickness of the muscle tissue in the bronchi of the asthmatic.

24. Bayliss: General Physiology, p. 538.

The deductions which are permissible from the data obtained by than 2 mm. outside diameter is greater in the asthmatic group. (2) these measurements are: (1) The wall thickness of tubes of more This difference is due to increased thickness of the subepithelial layer, of the muscle bundles and of the tissues outside the muscle bundles.

TISSUE EOSINOPHILIA IN BRONCHIAL ASTHMA

Of the cellular elements found in the bronchial secretory system and its product, the sputum, the most interesting are the eosinophil

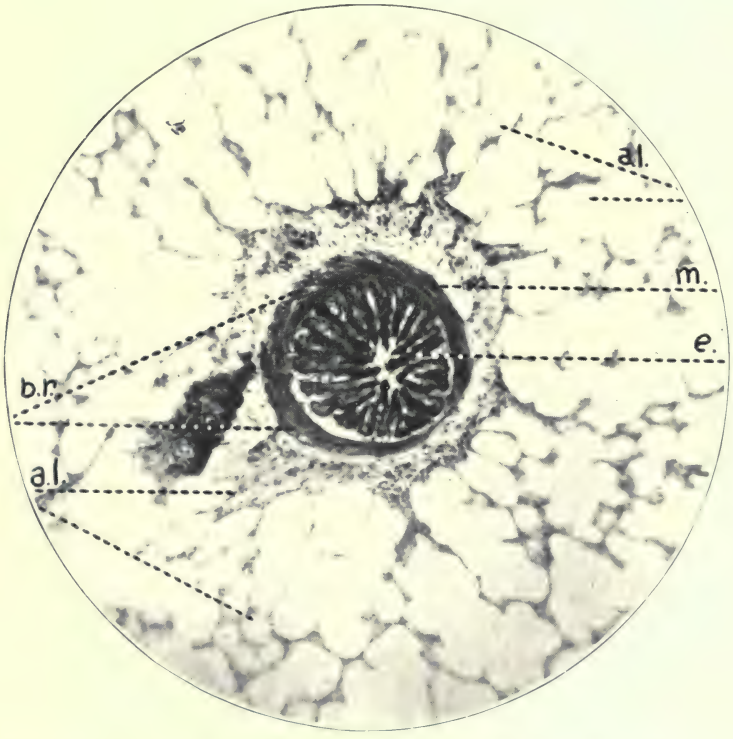

Fig. 19.—Guinea-pig. Fatal horse serum anaphylaxis. Bronchiole. Cross section. Lumen obliterated by folded epithelium. *e* = epithelium; *m* = muscle; *al* = alveoli. Magnification, 90 diameters.

cells. Their numerical increase in the circulating blood and their predominance in the sputum during the attack have been considered for many years a characteristic clinical symptom of asthma. Since the diagnostic value of this finding has been questioned, various observers reporting that in other diseases of the lungs and bronchi an increased number of eosinophils is present in the sputum,[25] we wish to state that

25. Von Hoesslin, H.: Das Sputum, Berlin, Springer, 1921, pp. 114-120.

TABLE 5—General Summary—

Case	Macroscopic Description	Content of Lumen of Bronchi	Epithelium	Basement Membrane	Subepithelial Layer
1 v. Leyden (1886)	Marked vesicular emphysema; bronchial mucosa reddened; small bronchi partially occluded by mucous masses	Small bronchi partially occluded; Mucus adherent to epithelium	.....	.....	.....
2 Berkart (1889)	Right apical adhesions; lungs emphysematous; masses almost occlude right main bronchus and one branch of left; smaller bronchi dilated and partially occluded; heart dilated and hypertrophic; moderate ascites	Masses composed of degenerating cylindrical epithelium; small bronchi contain detritus and fragments of Charcot-Leyden's crystals	Almost all denuded; one deep ulcer extends almost to cartilage	.....	.....
3 Schmidt (1892)	Tumor masses in left side of neck and extending into anterior mediastinum and upper lobes of both lungs; bilateral apical adhesions; lungs contain much blood; small bronchi contain masses of mucus	Many bronchi almost occluded by masses containing cells; these masses form spirals	Well retained	.....	Much dense C. T.; infiltrated by round cells
4 Fraenkel (1898)	Right apical adhesions; lungs emphysematous; nodular hepatization in lower lobe; bronchi contain masses which can be pulled out thread-like; walls of bronchi thickened; dilation of right and left ventricles	Mucous masses contain twisted threads, and epithelial cells which are greatly elongated; no eosinophils	High grade desquamation	.....	Thickened
5 Fraenkel (1900)	Middle-sized and small bronchi occluded by screw-shaped clots	Mucous masses completely occluding many bronchi and made up of layers containing elongated epithelium and eosinophil cells	Cells in small bronchi greatly elongated	.....	Groups of leukocytic cells; many eosinophil cells
6 Jezierski (1905)	Right lung pneumonic; bronchi of left dilated; mucosa hyperemic	Middle-sized and small bronchi of left partly occluded by mucus containing desquamated epithelium, neutrophils, eosinophils and lymphocytes	Fairly retained and infiltrated by round cells	.....	Infiltrated by round cells; elastic tissue increased; many engorged blood vessels
7 Jezierski (1905)	Fluid in both pleural cavities; left lung emphysematous	Small bronchi of left occluded by mucous plugs containing elongated epithelial cells, round cells, eosinophil cells	Mostly intact	Interrupted by accumulations of round cells	Contains many round cells
8 Ellis (1908)	Lungs emphysematous; right adhesive pleuritis; many bronchi occluded by greenish colored mucus	Most of smaller bronchi occluded by mucous plugs containing some degenerated epithelial cells, neutrophils and a few eosinophils; mucous plugs show spiral arrangement	Mostly intact in smaller bronchi; desquamated in larger	Hyalinized in the larger bronchi	Contains many polynuclear cells, some being eosinophil cells
9 Mönckeberg (1909)	Ascites; anasarca; bilateral adhesive pleuritis; mediastinal and peribronchial lymph glands enlarged; lungs air-filled; walls of bronchi thickened; lumen of most of smaller bronchi occluded by mucus; right heart enlarged, aorta narrowed	Small bronchioles empty; larger bronchioles occluded by mucous plugs containing desquamated epithelial cells and round cells; mucus shows spiral arrangement; Charcot-Leyden's crystals in larger bronchi	Mostly intact	Hyalinized and thickened in larger bronchi	Infiltrated by round cells and many eosinophil cells; contains diverticula-like protrusions of epithelium
10 H. Heizer (1911)	Lungs distended; many bronchi almost occluded by mucus; right ventricle thickened	Contains leukocytes and epithelium	.....	.....	Thickened by infiltrating cells and distended capillaries
11 Tichmeneff (1913)	500 c.c. fluid in left pleural cavity; left adhesive pleuritis; cavity in left upper lobe; mucopus in bronchi; ulcer in duodenum	Mucus containing round cells and leukocytes in many bronchi	Greatly elongated; hyperplasia	.....	Thickened; marked round cell infiltration
12 Marchand (1915)	Lungs emphysematous; occlusion of many small bronchi; slight hypertrophy of right heart	Plugs in bronchi composed of epithelium, round cells and crystals	Contains many goblet cells; infiltrated by cells	.....	Infiltrated by round cells; some eosinophil cells

—OF PATHOLOGY OF ALL CASES

Muscle	Mucous Glands	Fibrocartilaginous Layer	Blood Vessels	Nerves	Alveoli	Lymph Glands	Other Tissue
.....	.....	.....	.....	.....	Some dilated; most filled by a granular material containing many large cells		
.....	Mucous glands small and atrophic	.....	.....	.....	Emphysematous; some contain a fibrinous exudate		
.....	Mucous glands in active secretion	.....	Periarteritis obliterans in vessels of upper lobe	.....	Some dilated and some filled by mucus; no spirals; bronchopneumonic areas about some bronchi		
.....	.....	Thickened; infiltrated by round cells	Extremely dilated in mucosa of bronchi	.....	Some dilated, some contain blood	Large and pigmented	
Eosinophil cells between muscle bundles	Marked cellular infiltration; many eosinophil cells in glands	.....	Bronchial capillaries markedly distended	.....	Patchy emphysema; alveoli contain a few eosinophil cells		
.....	.....	Contains many round cells	Many newformed engorged blood vessels in walls of bronchi	Phrenic nerve contains degenerating fibers	.....	Numerous groups of round cells in wall of bronchi	
.....	.....	Contains many round cells	Surrounded in many cases by round cells	.....	No change	Bronchial glands anthracotic	
Not changed	Contains many mononuclear cells, some being eosinophil cells	.....	Vessels in bronchial well engorged by blood	.....	Many distended; some contain many eosinophil cells	.....	Not changed
Increased in lobular septae; increased in bronchi and larger bronchioles	Large	Contains many mononuclear and polynuclear cells	Pulmonary artery sclerotic; pulmonary veins thickened	.....	Some occluded by granular precipitate	Prominent lymphoid nodules in larger bronchi	
....	Greatly enlarged and filled by mucus	....	Distended	.....	Some distended	All enlarged	
Hypertrophied; infiltrated by round cells	Enlarged; infiltrated by round cells	.....	.....	....	Some contain leukocytes		
Infiltrated by round cells	Contain many round cells	.....	.....	.....	.....	Distinct nodes formed in walls of middle-sized and smaller bronchi	

TABLE 5—General Summary—

Case	Macroscopic Description	Content of Lumen of Bronchi	Epithelium	Basement Membrane	Subepithelial Layer
13 Marchand (1915)	Pulmonary emphysema; some bronchi almost occluded by exudate; bronchial mucosa swollen and reddish; peribronchial lymph glands enlarged	Arranged in layers and contains many polynuclear leukocytes; a part contain strands of fibrin	Well retained; a few goblet cells	Thickened and hyalinized	Contains many eosinophil cells, some lymphoid cells, and a few polynuclear leukocytes
14 Marchand (1918)	Lungs distended; smaller bronchi occluded by yellowish masses; left antrum of Highmore filled by secretion; walls of right and left ventricle thickened	Mucous masses in bronchi contain leukocytes, round cells and eosinophils; mucus forms spirals in smaller bronchi	.....	.....	Capillaries engorged; infiltrated by cells; many eosinophil cells
15 Kamehorn and Ellis (1921)	Left adhesive pleuritis; all other serous cavities contain fluid; left lung collapsed; right upper and middle lobes distended; tenacious mucus in middle-sized bronchi; hypertrophy of right ventricle	Mucus contains epithelial cells, leukocytes and a few eosinophil cells and is arranged in spirals	Mostly desquamated	.....	Capillaries engorged; infiltrated by mononuclear cells
16 Huber and Koessler (1922)	Coroner's inquest; report not available; lungs distended; most bronchi, except larger, occluded by mucous masses	Mucous masses in bronchi contain desquamated epithelium, round cells, neutrophil and eosinophil leukocytes; mucus in layers and twisted; some bronchioles contain many eosinophil cells	Partially desquamated	Thick and hyalinized	Infiltrated by many round cells and a few eosinophil cells
17 Huber and Koessler (1922)	Emphysema of lungs; bronchi contain a small amount of mucus	Mucus in small bronchi is scanty and contains only a few epithelial cells	Mostly intact	Not conspicuous	Not uniformly thickened; contains many dilated capillaries
18 Huber and Koessler (1922)	Peritonitis, right adhesive pleuritis, pericarditis; small amount of mucus in smaller bronchi	Small amount of mucus containing a few epithelial cells and a few eosinophils	Mostly intact; goblet cells numerous in large bronchi	Thick and hyalinized	Infiltrated moderately by cells; some eosinophils
19 Huber and Koessler (1922)	Right fibrous pleuritis; marked emphysema; cut surface of lungs dry	Smaller bronchi contain a granular precipitate	Mostly intact; many goblet cells	Thick and hyalinized	Cellular infiltration marked
20 Huber and Koessler (1922)	Abscess of left lung; pulmonary emphysema; pneumonia of right lung; bronchial walls thickened; hypertrophy of right heart	Contains fragments of epithelium and neutrophil leukocytes	Partially desquamated	Indistinct	Markedly infiltrated by mononuclear cells
21 Huber and Koessler (1922)	Moderate emphysema of both lungs; small firm nodules in both lungs; some bronchi occluded by mucus; lymph glands large	Contains fragments of epithelium, neutrophil and eosinophil cells	Mostly intact; some goblet cells	Not conspicuous	Contains a few round cells and eosinophil cells

on the basis of the examination of several hundred patients suffering from true bronchial asthma and other respiratory disease associated with dyspnea, we can say that the coincidence of sputum and blood eosinophilia in the same individual seems to be a pathognomonic symptom of the asthmatic state. Exceptions to this statement are pulmonary distomatosis and hydatid disease.

In our work we are mainly concerned with the local accumulation of eosinophil cells in the tissues of the bronchi a localization which has

—OF PATHOLOGY OF ALL CASES—(*Continued*)

Muscle	Mucous Glands	Fibrocartilaginous Layer	Blood Vessels	Nerves	Alveoli	Lymph Glands	Other Tissue
Tissue between muscle bundles contains round cells and some eosinophil cells	Large cells filled by mucus; gland duct epithelium contains many goblet cells	Contains many lymphoid cells	Distended and many contain numerous polynuclear neutrophils	.....	Some areas completely consolidated	Some small lymph nodules contain a few eosinophil cells	
Hypertrophied	Enlarged	.....	.....	.....	No change	One contains a small tubercle	
No change	.....	.....	Sclerotic				
Thickened	Enlarged; structure altered by many foci of round cells; infiltrated by eosinophil cells	Some cartilages calcified	No change	No changes	Irregular in size	Anthracotic	
Thickened	Small; contain many foci of round cells and areas of areolar tissue; ducts form distinct ampullae	No change	Walls of bronchial arteries thickened	No changes	Irregular in size; some contain a granular precipitate	Anthracotic	No changes
Thickened; forms a distinct ring in some bronchioles	Large and containing many foci of round cells; gland ducts form distinct ampullae	Ossified cartilages	Walls of bronchial arteries moderately thickened	No changes	Vary in size	No changes	No changes
Not prominent	Small and densely infiltrated by round cells	Calcified and ossified cartilages	Walls of bronchial arteries markedly thickened	No changes	Patchy obliteration of alveoli; these areas contain hyperplastic epithelium	No changes	Spleen contains many eosinophil cells
Structure altered by round cell infiltration	Small; acini separated and composed of compact masses of cells	Calcified areas in cartilages	Walls of bronchial arteries thickened	No changes	Obliterated in patches by pneumonic foci	No changes	No changes
Eosinophil cells between muscle bundles	Enlarged; contain foci of round cells and eosinophil cells	Many eosinophil cells between cartilages and muscle layer	Walls of bronchial arteries thick	No changes	Some areas of absorption atelectasis	Contain eosinophil cells	Spleen and thymus contain many eosinophil cells

been previously observed by others. This tissue eosinophilia is a phenomenon of far-reaching bearing, which it seems to us, if completely understood, would undoubtedly greatly elucidate the pathogenesis of asthma.

In the normal adult the bone marrow is the chief place of formation of the eosinophil cells (Ehrlich). This conception is substantiated by the frequency with which mitotic cell divisions of these cells are found in this locality. Additional evidence is furnished by the increased

number of these cells in the bone marrow in cases of high blood eosinophilia.[26]

From this viewpoint any increase of the eosinophils in the circulating blood and in the tissues is to be considered as a (protective?) reaction of the leukopoietic centers of the bone marrow to the stimulus of an unknown toxic substance. In bronchial asthma this eosinophilotactic substance is either actually formed in the tissues of the respiratory system or it has, wherever formed in the organism or wherever introduced to it from without, an elective affinity for this system. Such an elective organ localization of chemical substances is not to be considered as a special postulate for this problem but constitutes the basis of most pharmacodynamic actions.

There are, however, many histologists who consider the local eosinophilia in the bronchi as a true primary autochthonous tissue eosinophilia. They believe that the eosinophil cells are formed in the bronchial tissue through myelocytic metaplasia from the perivascular connective tissue cells over the "lymphocyte." The secondary blood eosinophilia may be due, then, to the passage of the eosinophil cells from the bronchial tissues through the bronchial capillaries into the general circulation, or it may be due to a simultaneous stimulation of both the pulmonic perivascular tissues and the bone marrow. The faculty of forming new myelocytic cells in the adult organism we usually concede only to those organs which in the development of the embryo possess the property of leukopoiesis, i. e., the bone marrow, liver, spleen, lymph glands, kidneys and thymus. In certain pathologic conditions (e. g., leukemia), and others associated with severe infections and toxemias (e. g., congenital syphilis) the remnant foci of the embryonic leukopoiesis in these organs are stimulated to proliferation and renewed activity in the direction of their original function. The difficulty that such preexisting myeloid tissue is not known in the bronchial mucosa and submucosa is removed by the assumption that all perivascular adventitial connective tissue is a latent hematopoietic and leukoblastic organ. This conception, based on the early studies of Virchow and later studies of Marchand on the cellular products of inflammation and on the perivascular lymphoma formation of Ribbert, has been especially adopted by Pappenheim, Weidenreich and by G. Schwarz.

As far as the bronchial mucosa in asthma is concerned, the idea of the autochthonous local eosinophilia is mainly based (1) on the occurrence of mononuclear eosinophil cells in the sputum and the bronchial tissue; (2) on the complete absence of such unicellular elements in the circulating blood, and (3) on the enormous number of the eosinophil

26. Opie, E.: Cells with Eosinophil Granulations, Am. J. M. Sc. **127**:217, 477, 988, 1904.

cells, polymorphonuclear as well as mononuclear, in the tissue, far too many, as is argued, to have been accumulated from the blood even if it showed a considerable eosinophilia.

The last quantitative argument might be first considered. It is well known that a very marked local tissue eosinophilia does frequently exist without eosinophilia of the blood. This is very often to be observed, for instance, in the periphery of malignant tumors, and then—a rarer incidence—in the pleura and exudate of eosinophilic pleuritis. It is just this discrepancy of the normal amount of eosinophils in the blood and of their abundance in the tissue which is taken as one proof for their local genesis. But it could be shown by a simple calculation that in bronchial asthma enough eosinophil cells can be removed from blood with a normal content of these cells to account even for their most extensive accumulation in the respiratory tissue. Thus Heineke and Deutschmann [27] observed during an asthmatic attack a decrease of the eosinophilic polymorphonuclear cells in the blood from 2.1 to 0.4 per cent. Calculated for 1 liter of blood, this means that 126,000,000 eosinophil cells have disappeared from the circulation and could have accumulated by chemotactic emigration in the tissues. J. G. Taylor, in his studies in leukemia, has made a similar calculation.[28]

What now regarding the occurrence of mononuclear eosinophilic cells ("myelocytes") in the tissues of the asthmatic patient?

Our material furnished us an unusual opportunity for studying this special phase of the question. The tissue eosinophilia was very marked in the first case, bacterial asthma (asthmatic bronchitis), in the fourth case, anaphylactic horse serum asthma, and excessively so in the sixth case—food asthma in an infant. The accumulation of eosinophil cells in the wall of and in the tissues about some of the bronchial capillaries is very striking but in the same section of the same bronchus the walls of and the tissues about many capillaries are free of eosinophil cells. Again, the location of the eosinophil cells varied greatly; in Case 6 they were most numerous between the muscle tissue and the cartilages, while in the other cases they were most numerous in the subepithelial layer. In some cases (Cases 1 and 6) the mucous glands contain many of these cells. In the wall of a blood filled bronchial vein (Case 6), which lies between a cartilage plaque and a muscle bundle, there are three migrating eosinophil cells, each of which lies between adjoining endothelial lining cells and partly inside the vessel. Immediately surrounding the vessel there are many polymorphonuclear eosinophil cells and a few small round cells. Many

27. Heineke und Deutschmann: Das Verhalten der weissen Blutzellen während des Asthmaanfalles, München. med. Wchnschr. **53**:797, 1906.

28. Taylor, J. G.: Studies in Leukemia, Contribution from the William Pepper Laboratory of Clinical Medicine, Philadelphia, 1900.

similar pictures are seen on further careful study of these tissues. The sinuses of the bronchial lymph glands (Case 6) contain many polymorphonuclear eosinophil cells but the sinuses of the mesenteric lymph glands contain only an occasional eosinophil cell. The spleen (Cases 4 and 6) shows very many eosinophil cells, almost all of which are polymorphonuclear.

Most of the eosinophil cells are polymorphonuclear cells with usually two or three nuclear fragments. On closer focusing and study, fine chromatin threads can be seen which connect the parts of the nucleus with each other. There are many cells which on first inspection give one the impression of possessing a single nucleus, but most of these, when studied in different planes of depth, with powerful magnification, are seen to have nuclei with two or three fragments. There remain, however, a few eosinophil mononuclear cells, whose nucleus cannot be separated by the eye in such a manner (Plate 3, C). These mononuclear eosinophilic cells are plainly not larger than the polymorphonuclear elements and are of the same type. Their nucleus is small, opaque, more deeply stained than in the polymorphonuclear cells immediately surrounding them, and its chromatin is very homogenous and pyknotic. The protoplasm surrounding this deeply stained nucleus is completely filled with acidophilic granules and no cells were observed where a transformation of granulopoiesis was suggested by a slightly basophilic stained protoplasm containing only a few eosinophilic granules. We could find no cells, however carefully we searched our preparations, which suggested a mixture of basophilic granulations with the eosinophilic as is found frequently in young alpha myelocytes. There was nothing to suggest transitional or intermediate stages between the many "lymphocytes" ("myeloic lymphoidocytes") and acidophilic cells. Nor were we successful in demonstrating any eosinophilic cells which showed evidence of mitotic division. There were no immature embryonic cell forms such as erythroblasts, promyelocytes or meta myelocytes or megakaryocytes. One would expect to find some of these cells if true myeloic metaplasia was taking place.

On the basis of these studies we reach the conclusion that these mononuclear eosinophil cells are not alpha myelocytes but degenerative forms of polymorphonuclear eosinophil cells whose nuclear substance has undergone a regressive metamorphosis. This conclusion became most suggestive when we studied the thymus in Case 6. Here we were able to find many large mononuclear eosinophilic cells with a loose structured, more pale nucleus, encircled by a wide protoplasm containing abundant eosinophilic granulations of varying size, i. e., true eosinophilic myelocytes. The presence of such cells in the thymus, of a

2-year-old child is a normal occurrence.[29] Autochthonous tissue eosinophilia in the thymus can be accepted as proved, for this organ contains active myeloid tissue and is, therefore, capable of metaplastic activation. Mitotic cell divisions, erythroblasts, eosinophilic as well as neutrophilic myelocytes have repeatedly been described in this organ.

Whether the mononuclear eosinophilic cells in the bronchial mucosa, as Schwarz [30] and also Marchand believe, are derived from the binuclear polymorphonuclear cells through amitotic direct cell division is impossible to decide.

Our studies, therefore, contain little evidence which would prompt us to abandon the view that the local tissue eosinophilia in the bronchial structure is due to emigration of alpha granulocytes from the blood in favor of the theory of their autochthonous ontogenesis from the perivascular tissue and transformation of the histogenous lymphocytes. The chemotactic toxin, fixed (or formed?) by the tissue of the bronchi, exerts simultaneously a stimulus upon the bone marrow in the direction of increased new formation of eosinophilic cells which through the way of the blood reach the bronchial tissue. The question of the nature of this eosinoplastic and eosinotactic toxin is closely allied with the physiologic and pathologic significance of the eosinophilic cells in general. Ehrlich [31] suggested nearly twenty-five years ago that eosinophils seem to appear especially in such places in the organism at which many cells are destroyed, especially cells of epithelial type (e. g., in carcinomatous tissue). There can be little doubt that certain relations exist between proteolytic digestive processes in the tissues and organs and the accumulation of alpha cells. Eosinophilia appears to be a response, a reaction to a stimulus produced by certain substances derived from the catabolism of proteins. It has been shown that the eosinophils increase markedly in the tissues of the intestinal tract during the process of digestion, but disappear completely in animals that have been starved.[32]

Neusser held that eosinophilia is produced in response to the increased activity of glands supplied by the sympathetic nervous system. Eppinger and Falta,[33] and their co-workers, on the other hand, see in

29. Schaffer, J.: Ueber das Vorkommen von eosinophilen Zellen in der menschlichen Thymus, Zentralbl. f. med. Wissensch., 1891, 401-417. Dudgeon, L. S.: A Contribuion to the Pathology of the Thymus Gland, J. Pathol. & Bacteriol. **10**:173, 1905. Fortescue-Brickdale, T. M.: Observations on the Thymus Gland in Children, Lancet **2**:1029, 1905.

30. Schwarz, E.: Die Lehre von der allgemeinen und örtlichen Eosinophilie, Lubarsch und Ostertag Ergebn. d. allg. Path. **17**: 1913.

31. Ehrlich, P.-Lazarus: Die Anaemie, Nothnagel's spec. Pathol., Ed. 1, 1898, p. 113.

32. Opie: Loc. cit.

33. Bertelli, Falta and Schwerger: Ztschr. f. klin. Med. **71**: 1907. Eppinger, H., and Hess, L.: Die Vagotonie, Berlin, 1910, p. 60.

eosinophilia a symptom of increased vagus tonus. They attempted to show that substances like pilocarpin, pituitary extract and nitrites, which increase the tonus of the autonomous nerves, produce an experimental eosinophilia.[34] Their work, however, could not be corroborated by Schwenker and Schlecht.

The whole problem of blood and tissue eosinophilia, in general, but especially in relation to bronchial asthma, reached an entirely new phase since it has been considered from the point of view of anaphylaxis.

There are some scattered observations in immunologic literature which suggest that blood and serum injections into animals are often followed by a considerable increase in the number of eosinophils in the blood.[35] But the true connection between eosinophilia and the anaphylactic process was first clearly recognized by Schlecht.[36] He had observed previously that serum injections were sometimes associated with a marked increase in eosinophilic cells in the blood and he ascribed this phenomenon to the introduction of the foreign protein. Later he and his collaborators,[37] found that eosinophilia developed regularly on reinjection of sensitized animals. He examined many proteins and their derivatives in this regard and found egg albumin, serum albumin and globulin, and especially, fibrin, efficacious in producing eosinophilia. Peptone acted less promptly and amino-acids (leucin, alanin, phenylalanin, glycin and asparagin) were without action. Schlecht and Schwenker discovered also the eosinophilic infiltration of the bronchi in the lungs of anaphylactic guinea-pigs and in the perivascular tissue in the edema of the Arthus phenomenon. On the basis of this work, eosinophilia is to be considered as a protective reaction of the organism against certain definite decomposition products of heterogenous and even homologous proteins. We are in complete agreement with

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34. Schwenker, G., and Schlecht, H.: Ueber den Einfluss sympathico- und autonomotroper Substanzen auf die eosinophilen Zellen, Ztschr. f. klin. Med. **76**:77, 1912. Bertelli, Falta and Schwerger: Ueber die Wechselwirkung der Drüsen mit innerer Sekretion S. Mittheilung über Chemotaxis, 1910, p. 23.

35. Stschastnyi: Histogenese der eosinophilea Granulationen, Beitr. z. path. Anat. u. z. allg. Path. **38**:456, 1905. Schwarz, E.: Loc. cit.,[36] p. 431.

36. Schlecht, H.: Ueber experimentelle Eosinophile u. basophile Leukocytose **27**: Congr. f. inn. Med., 1910. Ueber die Einwirkung von Seruminjectionen auf die Eosinophilen, etc., Deutsch. Arch. f. klin. Med. **2**:308, 1910. Ueber experimentelle Eosinophile nach parenteraler Zufuhr artfremden Eiweisses und über die Beziehungen der Eosinophilie zur Anaphylaxie, Arch. f. exper. Path. **67**:137, 1912. Ueber lokale Eosinophilie beim anaphylactischen Versuche, Verhandl. Congr. f. inn. Med., 1912, p. 416. Ueber allgemeine und locale Eosinophilie bei Ueberempfindlichkeit gegen organische Arsenpraeparate, München. med. Wchnschr. **60**:800, 1913.

37. Schlecht, H., and Schwenker, G.: Ueber lokale Eosinophilie in der Lunge anaphylactischer Meerschweinchen, Arch. f. exper. Path. **68**:163, 1912. Ueber die Beziehungen der Eosinophilie zur Anaphylaxie, Deutsch. Arch. f. klin. Med. **108**:405, 1912.

this point of view and believe that most clinical eosinophilias have to be considered as the reaction of a person in the state of allergy.

Probably the clearest example of protein sensitization in the human is pollen disease.[38] Morphologic studies of the blood of many patients lead us to the conclusion that blood eosinophilia is a constant symptom during the attack. In the months of the year in which the patient is free from hay-fever his eosinophilic cells in the blood are, as a rule, not increased. If the eosinophilia perists outside of the six or eight weeks of the hay-fever season and a few weeks following it, the patient is usually the subject of multiple sensitization, i. e., he is sensitized to other proteins, e. g., horse dander, or he is subject to eczema or urticaria, i. e., he is sensitized to certain food proteins. In the autumnal type of pollen disease due to the pollen of ragweed, sunflower and other compositae, the eosinophilia begins almost immediately on the day when the catarrhal symptoms begin and increases usually progressively until the third week. The asthmatic symptoms, however, usually do not become evident until from twelve to twenty days after the yearly resensitization with pollen has begun.

These clinical data are mentioned here in this connection because they show how the human organism acts in regard to the emigration of eosinophilic cells from the bone marrow into the blood on yearly parenteral introduction of a foreign protein. The eosinophilia of pollen asthma is a reaction of the organism sensitized to pollen protein. For most other types of asthma there exists no difficulty in accepting this point of view; they are cases of special protein sensitization either to food, animal or plant proteins. Less evident is the meaning of eosinophilia in the bacterial type of asthma, the eosinophilic bronchitis. Here the eosinophilic reaction in its relation to protein katabolism and allergy is not at once entirely evident. It is certain that bacterial protein possesses antigenic and anaphylactogenic properties and it would be a simple explanation to assume that the infective organism had sensitized the subject in the same manner as the pollen protein. This is a possibility. But it should be remembered that at least all acute bacterial infections, with the one exception of scarlet fever, are invariably associated with hypo-eosinophilia which, in the convalescence, is followed by hypereosinophilia. Aside from the eosinophilia, the evidence for the assumption that micro-organisms produce asthma through sensitization with their own body substance—the evidence for the existence of a true allergic asthma of bacterial origin—is very meagre. Skin sensitization tests with bacterial antigens made according to Woodehouse from stock cultures, as well as autogenous cultures, grown from

38. Koessler, K. K.: The Specific Treatment of Hay-Fever (Pollen Disease), Billings-Forschheimer Therapeusis of internal Diseases **5**:671-706, 1914.

the bronchial expectoration and including anaerobic as well as aerobic organisms, have proved of very little diagnostic value in our hands. After using them in many hundred instances we have scarcely three or four reactions which we could call positive.[39] Shall we conclude from this failure of obtaining skin reactions to bacterial antigens that all cases of bacterial asthma are in reality cases of true allergic asthma due to sensitization with an undiscovered protein, which we might discover if we would increase the scope of our tests? It is undoubtedly certain that the group of allergic asthmas will steadily increase with improved methods for the preparation of antigens, with increasing their numbers used in the tests and thus detecting new etiologic factors.[40] Yet on the basis of our clinical observations and studies we are led to believe that there remains still a definite group of cases of asthma, between 25 and 30 per cent. of all cases, which are of true bacterial origin. Blood eosinophilia in this group of cases is of exceedingly varying degree; it is sometimes very marked, sometimes slight and may even be absent completely.

The production of an eosinophilia through stimulation of the leukopoetic organs is not restricted to intact proteins of antigenic properties. Lower decomposition products of proteins, as albumoses and peptones, too, have the faculty of producing eosinophilia as well as bronchiolar spasm and arterial hypotonus with capillary stasis, the three cardinal symptoms which constitute the syndrome "asthma."

Bacterial asthma may thus be considered as being due to the products which micro-organisms form by their action on the proteins of the body's own tissues or on the proteins of the food in the intestinal tract. It is a peptone intoxication or amine intoxication (aminosis).

Other peptones and amines, while possessing bronchospastic activity, do not exert any eosinotactic stimulus upon the blood forming organs. The study of the formation of these toxic products (amines) by micro-organisms has been the subject of our investigations for many years. Our evidence for the relation of amines to asthma is, while not conclusive, very suggestive, and will form the subject of a special report. Here, we wish merely to point out that the production of amines of bronchospastic and hypotonic action, of which histamin is the chief representative, is mainly dependent on a particular type of otherwise common species of micro-organisms. Thus, of twenty-nine strains of *B. coli* studied, six were able to form histamin on a synthetic medium and no other chemical reaction, as fermentation of sugars, etc., could be

39. A detailed account of the clinical phases of our work will be published in the near future.

40. Cooke, R. A.: New Biologic Factors in Bronchial Asthma. J. Immunol. **7**:147. 1922.

found which would set this group of colon bacilli aside, except their faculty of decarboxylating amino-acids with the formation of amines.[41]

From our viewpoint, then, eosinophilia, if present, is a very important symptom of bronchial asthma. It is evidence of (1) the allergic type of asthma due to sensitization with proteins of antigenic character, or (2) of an intoxication with higher peptones. If constantly absent it forms, together with other clinical symptoms, strong evidence that the asthma is due to an intoxication with lower peptones or with amines or that the bronchospasm is produced by extraneous factors, pressure on the vagus by tumors or aneurysm (symptomatic asthma).

ABSORPTION ATELECTASIS IN BRONCHIAL ASTHMA

If the passage of gases (air) through a bronchiole or small bronchus is interrupted for some time, either through bronchiolar spasm or through obturation with secreted mucus, the air contained in the alveoli connected with that occluded tube is gradually absorbed by the blood of the pulmonary capillaries and an area of atelectasis is formed. In two of our cases, the infant with food asthma (Case 6) and the adult male (Case 4), who succumbed to an injection of horse-serum, definite areas of this character were shown. In the infant there are two distinct groups of areas scattered rather diffusely and which may be interpreted as representing two stages in the production of areas of absorption atelectasis. In one group of areas, apparently the newer stage, there is an obliteration of the alveoli by an exudate containing only a few cells, chiefly polymorphonuclear neutrophils. In these areas the bronchioles are completely occluded by a mucous exudate. In the other group of areas, apparently the later stage, there is such a complete obliteration of alveoli, that all vestiges of lung structure, except contracted bronchioles, are absent. Both of these areas are sharply defined and are separated from normal appearing alveoli only by the normal amount of fibrous tissue which separates the units of lung tissue in the child. We can think of these areas as representing two distinct end-results of different asthmatic attacks. In one, the newer, the occlusion of a small bronchus cut off temporarily that portion of the lung which connects with it and the exudation resulted; in the other, the older stage, the occlusion of a bronchus or bronchiole, occurred in a previous attack and lasted long enough so that complete organization of the lung connected with it resulted. In the adult (Case 4) we find also areas of lung tissue in which there is complete obliteration of all alveoli. In these areas, which are less sharply defined than in the infant's lung, there is a larger amount of fibrous tissue which surrounds, zonelike, all blood vessels

41. Hanke, M. T., and Koessler, K. K.: Studies on Proteinogenous Amines, XII. J. Biol. Chem. **50**:131, 1922.

and small contracted bronchioles. These areas we interpret as representing old lesions following previous asthmatic attacks. No other lungs, whether normal, asthmatic or nonasthmatic, showed such areas. The presence of absorption atelectasis has heretofore not been observed in bronchial asthma. Pathogenetically it is, like the acute emphysema, the result of the bronchiolar obstruction and represents the ultimate stage of the emphysema in an area of lung tissue in which the normal gaseous exchange through the bronchi has become completely interrupted.

Some authors [42] have attempted to set apart certain forms of paroxysmal dyspnea in children as a new morbid entity, which they call "bronchotetany." This, according to its originator (Lederer), is a symptom complex fundamentally distinct from bronchial asthma, though also due to bronchiolar spasm. The distinction is based first on the clinical observation that bronchotetany is found usually only in children who show some definite symptoms of spasmophilia, such as laryngeal spasm, carpopedal spasms or eclampsia, and second, on the evidence of the pathologic anatomy that all fatal cases of bronchotetany are associated with the formation of areas of atelectasis. For after studying the published reports of twelve postmortem examinations of bronchial asthma, all of which we have cited in our historical review, this investigator reaches the conclusion that "intense bronchospasm leads in asthma only to emphysema, never to the formation of atelectasis; in bronchotetany always to atelectasis." From the clinical description it is not clear to us how the cases cited as bronchotetany differ from true (allergic?) asthma in children. The presence of definite areas of absorption atelectasis in our two cases of bronchial asthma, shows that also the specific pathologic criteria evoked for the existence of this new entity, bronchotetany, do not hold true.

SUMMARY

The pathologic histologic examination of the finer structure of the bronchi and their branches in our six cases of bronchial asthma suggests a certain parallelism between the clinical picture and the structural changes. Of those cases developing seemingly on a bacterial basis, one was during life a well defined exudative type (Case 1), characterized by an abundant secretion (bronchorrhea) which was one of the chief clinical symptoms associated with the paroxysmal attacks. The anatomic parallel of this picture is furnished by the striking hypertrophy of the mucous gland system of the bronchi.

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42. Lederer, R.: Ueber Bronchotetanie, Ztschr. f. Kinderh. **7**:1, 1913, and ibid. **23**:79, 1919. Chronische Bronchitis, Bronchial Asthma und Bronchotetanie, Ergebn. d. inn. Med. u. Kinderh. **19**:564, 1921. Rietschel, H.: Bronchotetanie, Bronchialasthma und Asthmatische Bronchitis. Monatschr. f. Kinderh. **12**:261, 1913.

One other case of bacterial asthma (Case 2) was characterized clinically by a more or less unproductive cough which led to the attacks of bronchospasm. The chief pathologic changes seen in this case are the hypertrophy of the smooth muscle system and the atrophy of the mucous glands.

The two cases of food asthma (Cases 3 and 6) are characterized pathologically by hypertrophy of both glandular and muscular systems, both of which undoubtedly played a rôle in the production of the stenosis.

One case of true allergic asthma (Case 4) in a person hypersensitive to horse serum, calls to mind the picture seen in the lungs of the guinea-pig in the classical anaphylactic experiment, i. e., the acute emphysema and the marked contraction of the bronchi and bronchioli by the well developed muscle layer. The irregular thickening of the walls of the bronchial arteries recalls the nodal arrangement of these arteries described by Schultz as occurring in the guinea-pig.

The outstanding finding in our study is the evidence that the actual thickness of the walls of bronchi and of bronchioli of more than 0.2 mm. outside diameter is increased, as compared with similar structures in nonasthmatic persons. This difference is due to increased thickness of all layers from the epithelium to the outer fibrocartilaginous layer. Hyperemia and cellular infiltration of the wall and increased activity of the glands lead to swelling and thickening and this can produce, mechanically as well as chemically, irritation of the peripheral nerve endings in the tube, which may indirectly cause bronchospasm. The abundant secretion of the epithelium and the hyperactive glands obstruct, in some instances completely, the already narrowed lumen of the middle-sized and small bronchi and the bronchioli. In this way both systems, the exudative and the bronchomuscular, act simultaneously in the production of the stenosis, in some cases one more than the other but always both to some extent. Even in the purely allergic asthma of the infant 16 months old, which at that age already showed definite thickening of the bronchial wall as compared with an infant of the same age, the exudation into the bronchi and bronchioli with complete obstruction of some is proof of this combined involvement. These observations make it plain that in man, at least, the allergic reaction of the tissues is not confined alone to the smooth muscle fiber system, but involves also the whole organ system which serves exudative processes, endothelium, epithelium, capillaries and glands.

The increased thickness of the wall, the hyperactivity of the glandular system, the bronchoconstriction, as well as the emphysema, are not present to the same degree in all parts of the lungs but often involve one lobe or part of a lobe to a greater degree than others.

The anatomic substrate of the bronchospasm is mainly furnished by the hypertrophy of the smooth muscle fiber system. The evidence of a narrowed lumen and the folding of the epithelium while present has to be interpreted with great care.

The chief cellular symptom of the allergic reaction in man is the eosinophilia. In only one disease, bronchial asthma, does a blood, sputum and tissue eosinophilia occur simultaneously. The eosinophilic infiltration of the bronchial wall in asthma is a characteristic histologic criterion of bronchial asthma, but if absent it does not exclude asthma. Since eosinophilia is regarded as one of the chief clinical and pathologic symptoms of allergy, its constant absence in certain forms of bacterial asthma is regarded as one important part of evidence that there are types of asthma which may not be of allergic origin. This form may be regarded as due to an intoxication with peptones or amines, bronchospastic poisons, which are formed by the action of micro-organisms on tissues.

This work would not have been possible without the kind interest and aid of several colleagues. To all of them we wish to express our grateful appreciation. Especial thanks are due to Drs. H. G. Wells, T. H. Boughton, B. O. Raulston, W. White, F. H. Stangl, W. G. Hibbs and C. J. Eldridge for assistance in obtaining some of the normal and pathological material used in this study and also to Mr. K. Toda for his excellent colored drawings.

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THE TRANSITION FROM CRYSTALLOID TO COLLOID PROPERTIES WITHIN HOMOLOGOUS SERIES

BY EDGAR J. WITZEMANN[1]

The properties of colloidal and crystalloidal solutions are generally discussed from the standpoint of their differences and less frequently from the standpoint of their similarities.[2] The former is done for convenience in presentation; the latter because they really are quite similar and as Einstein emphasized in a mathematical treatment of the subject,[3] there is a gradual transition from crystalloid to colloid properties. There is in living organisms a special case of gradual transition from crystalloid to colloid properties within homologous series. This transition is not static but represents a dynamic equilibrium which is, so far, largely peculiar to the living organism and which seems to be an indispensable characteristic of life as we know it.

The biological aspects of the general problem of colloidal transition fall apart into three considerations.

(1) The factors that influence this transition in general with a given group of compounds.

(2) The condensation $\rightleftarrows$ hydrolysis equibrium by which the transition is made from one end of the series to the other.

(3) The biological factors that sensitize and speed up this equilibrium.

In this paper we shall discuss this problem of transition mainly with respect to the first of these considerations in order to determine whether the relations involved can be considered in simple terms of chemical constitution, for instance. It seemed likely that to consider all solutions as a continuity would aid eventually in understanding certain aspects of many

[1] Contribution from the Otho S. S. Sprague Memorial Institute, Rush Medical College, Chicago, Ill.

[2] Zsigmondy-Spear: "Chemistry of Colloids," p. 23 (1917).

[3] Einstein: Ann. Phys., (4) **17**, 549 (1905); **19**, 371 (1906); Zeit. Elektrochemie, **14**, 235 (1908).

biological problems such as diabetes mellitus for instance in which the organism can convert a colloid (starch) into a crystalloid (glucose) but cannot get it back again into the colloid state (glycogen) adequately.

The main components of living organisms (other than water) are the so-called biocolloids (lipoids, carbohydrates and proteins) and the simple molecules or crystalloids of which they are built up. These biocolloids as a group differ from other colloids in the degree to which they show certain typical characteristics of colloids. For instance they are in general more completely reversible than most other colloids. In fact they show two interesting kinds of reversibility.

(1) These colloids as a group may be taken out of or put into colloid-solution as often as desired, and in general if nothing is done to them that is incompatible with the life of the organism from which they are derived, they show little tendency to become irreversible. In any case the biocolloids are the type substances of the reversible colloids.

(2) In the living organism they exhibit a tremendous capacity for reversibility of the equilibrium represented thus:

$$\left\{\begin{array}{c}\text{Chemically labile}\\ \text{Physically stable}\\ \text{Crystalloids.}\end{array}\right\} \rightleftarrows \left\{\begin{array}{c}\text{Physically labile}\\ \text{Chemically stable}\\ \text{Colloids.}\end{array}\right\}$$

A large portion of the physical chemical philosophy of the cell is graphically summarized in this equilibrium. The chemical processes of life take place in chemically stable colloidal systems built up from crystalloids by virtue of chemical transformations of other portions of the same crystalloids.

The formulation of this reversible equilibrium visualizes some interesting paradoxical situations. Both sides contribute indispensably to the joint result—namely, life—but from the viewpoint of either side of the equation the material on the other side is playing a passive rôle. Its effects are indirect. Assigning "social" attributes to the molecules in question we have here a kind of chemical "altruism." Moreover the system contains both a dilute and concentrated solution since the

discontinuous phase contains a lower concentration of water (in biological systems) than the continuous phase. Therefore if condensation reactions thrive best in the more concentrated phase hydrolytic reactions should thrive best in the other. This discontinuity moreover gives rise to a differentiation in the energy components of the two phases the full consequences of which are yet to be appreciated and concerning which more will be said below.

These two types of reversibility so important to the chemical organization of life seem to fit these biocolloids peculiarly for their rôle in life processes as we know them. The chemist cannot aid materially in the dynamic interpretation of these processes until he can connect these two groups of spontaneous effects with the properties of the compounds in question and at the same time formulate his interpretation so that these transformations are seen to take place in accordance with the known principles of thermodynamics. Recent experience in the interpretation of the transformations of colloidal manganese dioxide[1] on the basis of equilibria sensitive to changes in temperature, in concentration and in the nature of the reagents suggested that perhaps the transition from crystalloid to colloid properties and *vice versa*, considered above, could be interpreted on the basis of chemical constitution. The results of such an inquiry are briefly stated in what follows.

The Fatty Acids

The commonest biocolloids constitute three complex homologous series—namely, the fats, carbohydrates and proteins—all of which are homogeneous or heterogeneous in their chemical components. For the sake of definiteness and simplicity it will be best to consider the fats first and these preferably in the form of their sodium and potassium soaps or salts. These compounds have the simplest chemical structure and within a truly homologous series show a strikingly definite transition from simple crystalloid qualities, in the lower members, to definite colloid properties in the higher members. The

[1] Jour. Am. Chem. Soc., **35**, 1079 (1915); **39**, 25 (1917).

main facts concerning this series will be recalled in connection with the brief review given in the following paragraphs.

(1) It is well known that after butyric acid the fatty acids rapidly become increasingly insoluble in water. Thus from complete miscibility in water for the lower acids there is a gradual passage to almost complete insolubility and immiscibility in the higher members.

(2) On the contrary the sodium and potassium salts of fatty acids are much more soluble, except the lowest members of the series, than the corresponding free acids. With increasing molecular weight the aqueous solutions tend to become more and more colloidal. Here, too, we have a gradually decreasing miscibility with increasing molecular weight manifested in a somewhat different way.[1] Thus the substitution of the hydrogen atom in the carboxyl group with potassium or sodium increases the range of solubility of the fatty acids and increases their capacity to take up water in the case of the higher acids in which colloidal solutions are formed.

(3) If we accept the main idea of the solvate theory of solution, namely, that solvent and solute interact in some way, then in the case of the fatty acid series the introduction of sodium or potassium in the carboxyl group increases the affinity of the molecule for water. Since a fatty acid is a constant (the carboxyl group) attached to a variable (the hydrocarbon group) it would obviously be unreasonable to expect the entire series of soaps to interact with water in exactly the same way. Now it is well known that water forms hydrates with formic and acetic acids and the alkali salts of lower fatty acids;[2] but the idea that these hydrates vary in complexity and stability for different fatty acids has not been much used as a working conception. Thus the long-known anomaluos volatility of the lower fatty acids[3] on distilling dilute aqueous solu-

[1] Cf., for instance, Mayer, Schaeffer and Terroine: Comptes rendus, **146,** 484 (1908).

[2] Jones: Carnegie Inst. Publications, No. **210** (1915); Cf. summary and list of earlier publications at the end. Turner: "Molecular Association" (1915).

[3] Duclaux: Ann. Chim. Phys., **2,** 289 (1874).

tions had no simple rational interpretation until recently the principle of decreasing stability of the fatty acid hydrates with increasing hydrocarbon function was used to supply one.[1]

(4) The idea that these compounds interact with the solvent is supported by many well-known lines of evidence only a few of which will be mentioned.

If the growing insolubility of the acids in water is due to the increasing significance of the hydrocarbon function and this group and the carboxyl act in an opposite sense on the solubility as may be concluded from much data in the literature[2] then the substitution of hydrogen in water with hydrocarbon groups should increase the solubility of fatty acids in the solvent. Now it is well known that soaps and fatty acids give true solutions with alcohols to a higher point in the series and to higher concentrations than they do with water. Moreover if the presence of the hydroxyl group in water is associated with the favorable effect of the carboxyl group on the solubility of the fatty acid, then the introduction of hydroxyl groups in the hydrocarbon group of a higher fatty acid should favor solubility of the fatty acid in water. That the hydroxy fatty acids are more soluble and interact more strongly with water is well known.

Other effects of the hydrocarbon depending on the character of the carbon chain, the number of carbon atoms in it and the position of the substituents, all have marked influences upon the solubility, but these factors do not modify the minimal statements given above concerning the influence of the two component groups of a fatty acid upon its general behavior toward water.

Physical and Chemical Theories of Solution

In order to make this discussion of the problem of the transition from crystalloid to colloid properties within a homologous series concrete it seems necessary to compromise the immortal controversy between the physical and chemical

[1] Witzemann: Jour. Am. Chem. Soc., **41**, 1946 (1919).

[2] For example cf. Auwers: Zeit. phys. Chem., **42**, 542 (1902).

theories of solution.[1] After what was said in the preceding paragraphs it seems clear that whether the one or the other theory most nearly expresses the facts will depend upon the chemical constitution of the solvent and the solute in question. Thus hydrocarbons in hydrocarbons give solutions in which the two components of the two systems of molecules differ quantitatively in composition but not qualitatively. We should here expect a minimum of chemical interaction and many of the properties of the solution should represent the algebraic sum of those of the components. Such solutions would best conform with physical theories. With water and hydrocarbons we have no chemical resemblance and have no way of knowing *a priori* how these two substances will interact. Having found that they do not interact to form a solution we have two possible ways of modifying the mixture in order to attain solubility. We may chemically make the hydrocarbon more like water by putting a carboxyl or hydroxyl group into its molecule or we may make the water more like hydrocarbon by replacing a hydrogen atom with a hydrocarbon group. Both systems now interact and will conform more or less closely with the physical or chemical theory of a solution depending upon the nature of the groups involved, *i. e.*, in which component the concession to the chemical constitution of the other was made.

As a qualitative statement the above is probably generally accepted. The difficulties arise in attempting to apply quantitative definitions and are partly due to the fact that our qualitative conceptions are not yet sufficiently clear. Moreover there is a tendency to set up discontinuities in our consideration of these phenomena where gradual transitions only exist. In this way imaginary boundaries are set up which we find ourselves more reluctant to cross as the data in the two fields multiply.

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[1] Cf. P. Walden; "Die Lösungstheorien in ihrer geschichtlichen Aufeinanderfolge," Ahrens Sammlung Chem. u. Chem.-Techn. Vorträge, **15**, Nos. 8–18 (1910).

In a recent study of water in the state of vapor, Oddo[1] pointed out that the more rarefied water vapor becomes, or the lower the vapor pressure, the greater the percentage of water dissociated into H^+ and OH^- ions. On the other hand Oddo also found that after a certain vapor pressure was attained the percentage of ionic dissociation continues to dcerease but that the percentage of molecular association rapidly increases. Oddo concluded that it seems that nature likes about so many particles of the constituents of water in a given volume and tends to break them apart or double them up in the attempt to maintain this number.

Jones[2] observed similar effects with mixtures of alcohol and water and acetic acid and water.

"An associated liquid tears down the molecules of an electrolyte dissolved in it, into simpler parts or ions; and it might be expected that such a liquid would tear down molecules of another associated liquid, a nonelectrolyte, not into charged parts or ions, but into simpler molecules. Acohol and water are associated liquids, as has been shown by the surface tension method of Ramsay and Shields. Do these diminish the association of one another?

"That this is the case was shown by Murray.[3] He worked with associated liquids, water, formic and acetic acids. He determined the molecular weight of each of these liquids in the other two and found that the molecular weight became smaller the more dilute the solutions. This showed that the solvent, i. e., the liquid present in the larger quantity, was tearing down the molecular complexes of the dissolved liquid or the one present in the smaller quantity."

Application of Le Chatelier's Principle

The above graphic statements of Oddo and Jones seem to resemble the qualitative statement of the second law of Thermodynamics known by chemists as the Theorem of Le Cha-

[1] Gazz. chim. ital., **45** I, 319 (1915).

[2] Carnegie Inst. Washington Pub., No. **210**, 163 (1915).

[3] Am. Chem. Jour., **30**, 193 (1903).

telier, by physicists as the Theorem of De Maupertuis or the Principle of Least Action, by biologists as the Law of the Survival of the Fittest, and by economists as the Law of Supply and Demand.[1] If this apparently universal principle governing natural phenomena can be adequately applied to a formulation of the phenomena of true and colloidal solution it would constitute a simplification of the problem and an advance at the same time. The example cited by Jones will serve to discuss the proposition concretely and thus to test the applicability of this Principle of Le Chatelier of the problem of solution.

Pure liquid acetic acid and liquid water are largely dimolecular. When a little of the acid is dissolved in the water dimolecular acid and water break up and acetic acid hydrate is one of the products. The total number of dimolecular molecules is less than the sum of the dimolecular molecules orginally present in the two component liquids. This distribution of acetic acid in water thus really involves two effects. (1) It involves the tendency on the part of the acid to distribute itself in the volume available as though it alone were present. This involves the breaking up of some associated molecules and some ionic dissociation analogous to what Oddo pictured for water. (2) It involves the interaction of these components of the acid system with the components of the water already present and this involves the formation of the various ion and molecule hydrates. The whole system of changes can be expressed in a series of equilibria[2] and the final condition of the system undoubtedly conforms with the Second Law of Thermodynamics, of which Le Chatelier's Principle is the qualitative statement.

When additional acid is added, the whole cycle of changes and adjustments is repeated until presently enough acid has been added so that it becomes obvious from molecular weight determinations such as those cited above that a smaller

[1] Paraphrased from a paper by W. D. Bancroft: Jour. Am. Chem. Soc., **33**, 91 (1911)

[2] Cf., for instance, Witzemann: Jour. Am. Chem. Soc., **39**, 25 (1917).

proportion of dimolecular acetic acid molecules is now being broken up. Into such a solution we could pass monomolecular acetic acid vapor and find it in the aqueous solution largely as dimolecular acid. Such a solution presents three kinds of acetic acid—ionized acid, monomolecular acid, and dimolecular acid—all in equilibrium with each other. The system is a completely reversible and reproducible one and may be prepared from any of the three acid components and water by adjusting the concentration of the water. Whether all three kinds of acid were present in the solution when equilibrium was attained after the first addition is probably not certainly known, but that as the concentration increases the dimolecular kind of acid increases faster than the other two kinds is clearly indicated by much experimental data. Since all of these kinds of acid are freely soluble in water the system is commonly considered to be homogenous. That it is not truly homogeneous is obvious.

The particular heterogeneity in which we are interested at this point is that involved in the formation of dimolecular acid from and in the presence of monomolecular acid within the aqueous solution. Here we have the spontaneous formation of a more condensed system within a less condensed one. Or using colloid chemical terminology a discontinuous phase (or concentrated solution of the colloidal substance) within a continuous phase (a dilute solution of the colloidal substance in the solvent) What may appear to be an unimportant distinction in the case of acetic acid as discussed ceases to be so if we take the sodium or potassium salts of the lower and higher fatty acids as well. One of the best-known facts of colloid chemistry is the fact that in passing up this series the significance of the ionized and monomolecular molecules diminishes more and more while the typical colloidal polymolecular molecules dominate more and more. Here we have developed for identical molecular concentrations of various salts the series of effects observed with variable concentrations of acetic acid.

The discussion in the preceding paragraphs confirms the

impression that the process of formation of both true and colloidal solutions, although not understood in its details, is obviously amenable to Le Chatelier's Principle. The molecular state of the fatty acids and their salts in solution presents no sharp break but rather a gradual transition from a less obvious to a more obvious heterogeneity. This change is seen to coincide with the increase in the hydrocarbon function of the molecule and since the other chemical function (carboxyl) is constant for the series the change must be definitely attributed to the constitutive influence of the hydrocarbon group. Consequently the decreasing dispersibility of these higher fatty acid salts is to be attributed to the increasing hydrocarbon function. Because of the chemical simplicity and stability of this group these salts and their derivatives should be expected to play the important biological rôle that they do.

Differentiation of Energy in the Two Phases

The fact that the soaps are not uniformly dispersed in aqueous solution but that the solution represents a concentrated solution suspended in a dilute one gives rise to an unequal distribution of energy between the two phases of the solution. The situation from the standpoint of energy is analogous to the behavior of compressed carbon dioxide. When the pressure is released the kinetically cool gas escapes into a warmer atmosphere. In expanding a portion of the gas is heated while the remainder is cooled. The end products are relatively warm carbon dioxide gas and cold carbon dioxide snow. A marked heterogeneity in the energy content of carbon dioxide was produced on releasing the relatively homogenous carbon dioxide into a similarly homogeneous atmosphere. A singularly parallel stepping up and down of the energy content of the component molecules of a quantity of soap occurs when it is dispersed in water. In the case of soap, however, we obtain a differentiated system that is stable in comparison with the carbon dioxide system. Since differentiation of heat energy content as in the case of carbon dioxide gives rise to unstable systems in which energy equalization occurs rapidly

if unhindered, it is obvious that the energy differentiation induced in these colloidal systems involves other kinds of energy than heat energy. The electrically charged colloidal particles (in so far as they are not due to ions) and the surface energies so pronounced in the colloidal state represent at least two known types of differentiation of other energies that are involved here.

This heterogeneity of energy distribution in colloids is conditioned by the orientation of the molecules within the system as a whole and manifests itself as surface energy only at the boundaries of the phases, as is shown by the interesting results of Harkins[1] for instance. These effects are particularly striking with simple compounds like the alkali soaps, the molecules of which have two ends of widely different chemical constitution. The same type of orientation probably is not conspicuous in the polysaccharides and proteins in which the active groups substituted in the hydrocarbon residues are rather evenly distributed through the large molecule.

Since the amount and kind of energy in the environment of molecules is an important factor in determining chemical reactivity, it is clear that such spontaneous differentiations of energies in colloidal systems would be desirable in the chemical organization of life because it would contribute so much to the chemical versatility of the organism. To have chemically cold, moderate and hot regions within submicroscopic distances of each other would be a great engineering advantage when the diversity of the chemical needs and processes of an organism are considered.

In summarizing for the fatty acid series we may say that increasing hydrocarbon function in this series gives rise to an increasing tendency to form two solutions in water at the same time (a concentrated and a dilute one) and that this material differentiation is associated with a differentiation in the amounts and kinds of energy present in the two phases of the

[1] Jour. Am. Chem. Soc., **39**, 541 (1917).

solution. Moreover it is clear that these transformations are taking place in accordance with Le Chatelier's Principle. Such characteristics as these, together with the fact that these systems are self-reproducing from the components, admirably fit these compounds as biocolloids.

The Biocolloids Proper

In the above we have traced the transition from crystalloid to colloid properties in a simple homologous series of compounds. The even more typical biocolloids such as the hexose—polysaccharide series and the amino acid—polypeptide—protein series are not so simply constituted.

Both series are built by condensation reactions from simple crystalloids and thus show the same gradual transition from crystalloid to even more characteristic colloidal properties than are observed with the soaps, in passing up the series. Both series may be considered as substituted hydrocarbons and their differences may be ascribed to the degree and nature of this substitution. In the carbohydrate series the substitution consists mainly in OH groups and the compounds of the entire series are in general feebly acid in nature and generally not highly reactive chemically except under special chemical conditions. The proteins on the other hand are built up of crystalloid components in which the basic amino group balances the acid carboxyl group. When the protein molecule is synthesized from a variety of these compounds with a hexose, purine, phosphoric acid molecule, etc., introduced here and there we have *a priori* a system that will be chemically temperamental or stable, depending upon the quantitative chemical nature of its environment.

The constitutional reason for the colloidal state in the higher carbohydrates and proteins is not exactly the same as that given in the case of soaps. In the soaps we had but two groups—the hydrocarbon and the carboxyl groups—which, according to Auwers' classification of groups with reference to their effects upon molecular association,[1] have an opposite

[1] Zeit. phys. Chem., **42**, 542 (1902).

effect upon molecular association of compounds in which they are substituted. In this series the increasing hydrocarbon function acts to decrease the affinity of the fatty acid for water and to increase its affinity for molecules of its own kind. In the case of the carbohydrates the increasing function is approximately CHOH of which group the OH, according to Auwers' classification, has distinct tendencies to facilitate molecular combination with other OH containing compounds, such as water. We therefore have greater solubility of the carbohydrate compounds than is shown by the corresponding hydrocarbons. That starch and cellulose are not freely soluble in water, however, shows that an unlimited number of OH groups does not secure unlimited solubility. In fact the facts in this connection are perhaps the best proof that the solvent interacts with the solute. It is a well-known fact that polyhydroxy compounds have a tendency to undergo condensation reactions by which two molecules react with the splitting out of a molecule of water giving rise to many simple and mixed complexes. Such condensations are however not limited to the kind just described. Anhydrides of some of these compounds are under some conditions more easily formed than the condensation products mentioned above. This is particularly true with γ-hydroxyorganic acids which give lactones so easily. The known chemistry of cellulose, for instance, is at present best explained by considering it as an anhydride of the γ-oxido form of glucose.[1] Compounds that so readily give rise to lactones and inner anhydrides have little tendency to add water at these points. In interacting with water they have a greater tendency to interact by means of their free OH groups to form hydrates than to add water at the anhydride oxygen rings and linkings. That is, the secondary valences of oxygen are more unsaturated toward water under these conditions than the anhydride linkages.

The same problem may be considered concretely in another way. The polysaccharide molecular complex contains many

[1] Hibbert: Jour. Ind. Eng. Chem., **13**, 256, 334 (1921).

OH groups the secondary valence energy of which is saturated off by adjoining OH groups in the same complex. When this complex network of OH groups interacts with a simple set of OH groups like that of water $(H_2O)_2$ the latter becomes molecularly dissociated and bound by the complex as hydration water without markedly dispersing the complex polysaccharide as a whole. Only as the final result of many such interactions with water molecules does a higher polysaccharide become dispersed in such solvents as water. Such a complex polysaccharide as starch should therefore be expected to swell and disperse slowly in water.

The process becomes still more concrete when pictured in terms of such simple electronic conceptions of valence as that of J. Stark,[1] details concerning which need not be given here.

In the protein molecule the changing functions, besides the hydrocarbon part, are the NH_2 and CO_2H residues which according to the same classification of Auwers also tend to facilitate molecular association with water. On the basis of the chemical nature of these groups the protein molecule interacts with water in some respects like the higher carbohydrates, but because the structural variety of groups in proteins is much greater the possibilities for sensitive influences become much greater. There is however no question about the gradual transition from crystalloid to colloid properties on passing up and down the series. Moreover it seems clear that if the above statements are of value in clarifying the relations in the fatty acid and carbohydrate series they are of value in the protein series although the relations are still somewhat more obscure on account of the more complex structure of these compounds and our ignorance of the influence of these various structural factors upon the properties of protein.

General Résumé

One of the most persistent problems of biological chemistry is the question as to how such chemical systems as con-

[1] Cf. P. Ruggli: Ahrens Sammlung Chem. u. Chem.-Techn. Vorträge, **19** (1912–3) for a review and refs.

stitute living organisms can come about. It is only as this question can be answered more or less clearly in chemical terms that the chemical dynamics of life processes become more amenable to attack with the conventional tools of chemistry. The above paragraphs constitute a partial answer to the question and state pretty clearly in terms of chemical constitution why the indispensable heterogeneity of the aqueous systems constituting living organisms exists and how it is spontaneously brought about. It is shown that the emulsoid structure of biocolloids constitutes a gross heterogeneity which is also present in a much less conspicuous form in the lower crystalloid members of the same homologous series. In this way it becomes clear that the particular properties of biocolloids arise from the cumulative effect of certain groups in the molecule. This effect of chemical constitution is especially easy to trace with the soaps and less easy with the carbohydrates and proteins.

The discussion also brings out the fact that there is associated with this material heterogeneity a well-known heterogeneity of energy. It is shown on the basis of a simple example how such heterogeneities may arise spontaneously upon releasing one system into another. The best known energy differentiation under these conditions is the much-discussed surface energy. In this discussion it appears that the surface energy phenomena in solutions are energy differentiations definitely associated with material differentiations and these in turn are due to chemical constitution and that changes in the latter necessarily involve the whole series of effects. Even the earliest students of chemical constitution knew that changes in constitution involved changes in solubility and therefore in the secondary effects of solubility. We however have been slow in following their lead particularly in considering the biocolloids.

So far as biological phenomena are concerned these two heterogeneities have in the past been regarded as associated with some vital force. But even now, when the aid of a hypothetical vital force is not so often called upon, these hetero-

geneities are frequently thought to be maintained in organisms by a constant expenditure of energy obtained from chemical reactions in the organism. Considerations developed in this paper indicate that certainly part of this heterogeneity occurring in the structure of organisms arises from a predisposition on the part of the component substances themselves to give rise to these effects. Such colloidal systems as those prepared from simple biocolloids are self-reproducing from the components: they arise spontaneously and require no unusual outlay of energy for their construction and maintenance.

Wm. Ostwald[1] has said that the two known laws of thermodynamics will take on a new comprehensiveness when we have learned to include the phenomena of biology among the data covered by what is still called thermodynamics. It is therefore particularly interesting to find that the qualitative statement of the second law known as Le Chatelier's Principle is so easily rediscovered in the phenomena of true and colloidal solutions so fundamentally involved in biological phenomena. This leads one to assert, whatever the errors made above may be, that the fundamental idea is right, since the most comprehensive statement yet developed concerning natural phenomena is so readily discovered to be operative in these phenomena of solution when the data are considered in this way.

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[1] Cf., for instance, "Der energetische Imperativ," pp. 12–13 (1913).

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THE ISOLATION OF A SUBSTANCE FROM URINE HAVING PROPERTIES OF CITRIC ACID: DESCRIPTION OF AN APPARATUS FACILITATING THE WORKING WITH SMALL VOLUMES OF GAS

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The normal urine of human beings yields pentabromacetone when treated by the method used by Kunz (11) for the determination of citric acid in wines. Briefly the method is: Potassium bromid and sulfuric acid are added to urine and the mixture heated to just below 55°C. A solution of potassium permanganate is added slowly. When the reaction is completed the solution is cleared with ferrous sulfate. A crystalline precipitate of pentabromacetone is deposited. The method is fairly accurate for quantitative determinations of citric acid, if a correction is applied. Amberg and McClure (2) found that if a volume of 50 cc. is used it is necessary to add about 5.5 mgm. citric acid ($C_6H_8O_7.H_2O$) to the end result. Urine also gave a positive test when subjected to the treatment used by Salant and Wise (14) for the demonstration of citric acid in urine. This treatment makes use of the Denigès reaction (6), (7). A solution of citric acid is heated after the addition of a solution of mercuric sulfate in sulfuric acid. The addition of potassium permanganate drop by drop to the hot solution causes the formation of a white precipitate. Acetonedicarboxylic acid, formed by the action of the potassium permanganate on citric acid, in the presence of nascent bromine is transformed to pentabromacetone while in the presence of mercuric sulfate it forms an insoluble mercury compound. The results of Amberg and McClure indicated the presence of a substance in urine which readily yields acetonedicarboxylic acid. None of the substances hitherto known to occur in urine had been shown to possess this property. Because acetonedicarboxylic acid is a characteristic product of citric acid cleavage and because citric acid, a normal constituent of milk of the human being, is not foreign to

the organism, it was concluded that the acetonedicarboxylic acid was derived from citric acid. The proof was not absolutely unquestionable and the present study was undertaken to substantiate further the conclusion by the isolation of citric acid with the formation of characteristic salts and by elementary analyses. In this we were not successful, but the results of our experiments support the conclusion that citric acid is a constituent of the urine of normal human beings. After a number of preliminary experiments the following procedure for the attempted isolation of citric acid was adopted.

If the urine is strongly acid its acidity is reduced to weakly acid reaction (litmus) with sodium hydroxid. Lead acetate is added to slight excess and then ammonium hydroxid to strongly alkaline reaction. The mixture is repeatedly stirred for about 12 hours when the precipitate is filtered off and washed by being taken up in water alkaline with ammonium hydroxid. The filtrates are evaporated over a free flame to convenient volume; the reaction becomes acid during evaporation. The solution is freed from lead by treatment with hydrogen sulfid and the filtrate and washwater from the lead sulfid is evaporated to a syrup, with loss of the excess hydrogen sulfid. Some crystalline material separates which is filtered off and freed from adhering mother liquor by washing with ice-cold water. The precipitate is discarded. Barium acetate (30 per cent) is added to the thin syrupy solution until no more precipitate is formed. Sometimes barium hydroxid was added to weakly alkaline reaction, but this was not found to be of special advantage. After the addition of 95 per cent alcohol, two to three times the volume, the precipitate is allowed to settle. The supernatant fluid is syphoned off, the precipitate is repeatedly washed with 50 per cent alcohol, and is finally collected on a filter. The precipitate is extracted two or three times with hot water acidified with ortho phosphoric acid, and filtered off after each extraction. The filtrates are evaporated to a small volume and treated with moderate excess of sulfuric acid. Plaster of Paris is added, and the evaporation continued to dryness. The residue is divided as finely as possible with a spatula and extracted with ether in a Soxhlet apparatus, in which the ether is renewed several times. The ether extract is evaporated and the residue taken up in water and filtered from some sticky material which is discarded. The filtrate contains phosphoric and sulfuric acids. Therefore, lead carbonate is added in as slight excess as possible, the liberated carbon dioxid is boiled out and ammonia is added to make the solution about 2 per cent. The mixture is left

standing a day or more, repeated shaking being necessary, or it is heated with ammonia, filtered, and the precipitate taken up several times in ammoniacal water and filtered. On evaporation the solution becomes acid, and it is free from phosphoric and sulfuric acids. The lead is removed with hydrogen sulfid and the filtrate from the lead sulfid is evaporated to dryness. The residue is redissolved in water and filtered from a small amount of undissolved material. The color of this solution is usually light yellow; sometimes it is rather highly colored. If this is the case, it is heated with a small amount of animal charcoal. This is to be avoided if possible, since animal charcoal absorbs citric acid. The strongly acid solution is neutralized approximately with sodium hydroxid and treated with barium acetate solution until no more precipitate forms. The precipitate is collected and washed with graded dilutions of alcohol. This precipitate is the crude product.

From an accumulation of urine 6.8 grams of this crude product were obtained. Calculated from the amount of pentabromacetone obtained from part of it, it contained at least the equivalent of 1.6 grams of citric acid; that is, about one-half of it may have been barium citrate.

For further purification the crude product is again extracted with water acidified with phosphoric acid. The filtrates are evaporated to a small volume and 5 per cent solution of mercuric sulfate in sulfuric acid is added until no more precipitate is formed. The precipitate is discarded. Mercury is removed from the filtrate by treatment with hydrogen sulfid, excess of which is removed by boiling, the mercuric sulfid is filtered off and the filtrate is treated with lead carbonate as before. On evaporation, the ammoniacal filtrate becomes acid and a small amount of white precipitate separates. This is collected separately (A), and separated from the filtrate (B). (A) is washed well with water, taken up in water, and treated with hydrogen sulfid. The filtrate is freed from hydrogen sulfid by evaporation and the residue is taken up in water. On the addition of barium chlorid to the acid solution, some precipitate forms. Barium chlorid is added very carefully until the solution does not give any further precipitate, nor react with sulfuric acid. The precipitate is filtered off, the filtrate evaporated to dryness and the residue extracted with alcohol. On evaporation the alcoholic solution becomes a syrup which leaves very little residue when taken up in water; the residue is removed by filtration. To the strongly acid solution barium acetate is added. A snow-white precipitate forms, dissolves, and reforms on addition of more barium acetate, as happens on addition of barium acetate to citric acid.

Dried in a desiccator over sulfuric acid this white precipitate obtained from the crude product weighed 101.8 mgm. On drying at 163°C. it lost 7 mgm. of water. Ninety-four and eight-tenths milligrams of the dry substance yielded on analysis 79.5 mgm. barium sulfate which corresponds to 89.7 mgm. of dry barium citrate and it gave an equivalent amount of pentabromacetone.

The bulk of the filtrate (B) from the lead carbonate is evaporated further and more precipitate forms. The lead is removed from the precipitate suspended in the solution and the filtrate is evaporated to a small volume. A solution of barium chlorid carefully is added until no more precipitate forms. The filtrate from this precipitate is evaporated to dryness, taken up in water, filtered from a small residue and precipitated with barium acetate. After the precipitate was washed with water, dilute alcohol and strong alcohol, it weighed approximately 0.6 gram.

There is scarcely a step in the procedure which does not entail some loss, a conclusion drawn from the positive Denigès' reactions of the filtrates and washings. The substance used for the analyses was not quite pure, but we decided to use it rather than to lose it all by further efforts at purification.

It is known that citric acid heated with strong sulfuric acid gives off carbon monoxid, molecule for molecule. If the heating is done in a current of carbon dioxid, the carbon monoxid can be collected over potassium hydroxid permitting a quantitative determination of citric acid (16). If a substance gives pentabromacetone and carbon monoxid in equivalent amounts, it may be assumed that this substance is citric acid. The carbon monoxid determination was carried out as follows:

The dry substance is placed in a small Erlenmeyer flask and is moistened with water. The flask is closed with a three-hole rubber stopper, connected by one glass tube to the carbon dioxid generator, and by another to a gasometer charged with 50 per cent potassium hydroxid. From a small separatory funnel, inserted through the stopper, concentrated sulfuric acid is dropped into the flask which is then heated on a water bath over an asbestos plate; the liberated gases pass into the gasometer with the current of carbon dioxid. One to two hours after the collection of the gas, the volume is read; the gas is then transferred to an absorber filled with ammoniacal cuprous chlorid solution, and the carbon monoxid absorbed. The gas remaining is transferred to a measuring apparatus filled with water, in order to determine the necessary correction.

For our purposes an apparatus was devised to facilitate the handling of small volumes of gas without loss (fig. 1). The gas liberated in the reaction is collected in a nitrometer over 50 per cent potassium hydroxid. When the gas is to be transferred to the absorber, the capillary glass tube adjoining the stopcock of the nitrometer is filled with water. A short gasometer is fitted to this glass tube with a rubber stopper. Leading off from a small trough at the bottom of this gasometer is a side tube to which the rubber tube of a levelling bulb is attached. This gasometer is filled with ammoniacal cuprous chlorid solution from the levelling bulb, which is then lowered. The gas is transferred to the absorbing gasometer with the levelling bulb of the potassium hydroxid gasometer. The apparatus is sufficiently elastic to permit considerable shaking. When the absorption is complete a second short gasometer filled with water can be attached, and any remaining gas can be transferred and the volume read. The apparatus could easily be modified to meet special demands.

Fig. 1. Apparatus devised for the determinations of small amounts of carbon monoxid.

For the determination of carbon monoxid 0.1736 gram of the air dry barium precipitate was used, giving 0.0107 gram of carbon monoxid, an amount equivalent to 80.3 mgm. of citric acid (calculated $C_6H_8O_7.H_2O$). In determinations of pentabromacetone the Gooch crucibles containing the pentabromacetone precipitates were weighed after drying 24 hours in a desiccator over concentrated sulfuric acid. The pentabromacetone was washed from the crucibles with acetone, the crucible was dried and weighed again and thus possible errors from acetone insoluble impurities were avoided. For this determination 0.143 gram of the purified substance was taken; the yield of pentabromacetone was equivalent to 65.3 mgm. of citric acid.

It has been stated that the barium precipitate does not represent pure barium citrate. Before preparing the pentabromacetone the barium was precipitated and 111.6 mgm. barium sulfate were obtained. This amount is equivalent to 136 mgm. barium citrate, with $3\frac{1}{2}$ molecules water of crystallization. The pentabromacetone obtained corresponds to 132.6 mgm. of barium citrate, while 143 mgm. of the barium precipitate was used. The carbon monoxid is equivalent to 163.1 mgm. barium citrate, while 173.6 mgm. of the barium precipitate was used. The amount of citric acid which should be present in 173.6 mgm. of the barium precipitate calculated from the amount of pentabromacetone found in 143 mgm. of the substance is 79.3 mgm., while with the carbon monoxid method 80.3 mgm. were found. The remaining part of the barium precipitate was dried at 164°C.; 119 mgm. yielded 8.16 mgm. carbon monoxid, an amount equivalent to 61.2 mgm. of citric acid; 127 mgm. yielded an amount of pentabromacetone equivalent to 63.2 mgm. citric acid. The amount of citric acid to be expected in 119 mgm. of material is 59.3 mgm. calculated from the pentabromacetone obtained from 127 mgm., while with the carbon monoxid method 61.2 mgm. were found.

Some test experiments with barium citrate dried at 164°C. are tabulated:

	BARIUM CITRATE	CITRIC ACID	
		Calculated	Determined
	mgm.	*mgm.*	*mgm.*
Pentabromacetone method	177.6	94.4	93.9
	191.5	101.8	101.5
	170.7	90.8	89.7
Carbon monoxid method	131.3	69.8	70.4
	109.8	58.4	58.2

The results obtained with the material isolated from urine show a rather good agreement between the amounts calculated and those found, taking into consideration that the pentabromacetone method may give values a little low and the carbon monoxid method values a little high. It is questionable whether the result of the second determination, not so good as that of the first, is owing to errors of method entirely. Our substance was not quite pure, and while pure barium citrate does not change when water of crystallization is driven off, an

impurity in our material may have undergone some change; which might account for the discrepancy in the results.

Our material was sufficiently free from organic impurities that on heating with concentrated sulfuric acid the color remained pale yellow, as in control experiments with citric acid or citrates. With material less pure the fluid becomes dark or even black.[1]

The results were obtained with material from one accumulation of urine; not enough material was obtained from other accumulations for similar analyses, because efforts at purification resulted in loss.

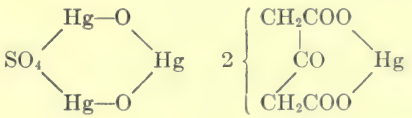
Only a few efforts were made to isolate our substance in the form of a calcium salt. It was very difficult to obtain any crystalline precipitates and if such occurred they were insignificant in amount. The difficulties here are as great as those encountered in efforts to isolate lactic acid from urine as a zinc salt. When calcium carbonate was added in place of barium acetate and the carbon dioxid was removed by heat, the neutral filtrate became acid and a small amount of a heavy crystalline precipitate separated rather early in the evaporation. This precipitate gave pentabromacetone and a positive Denigès' reaction. In one instance about 90 mgm. of this precipitate was obtained.[2]

With a small amount of good material the reaction of Sabanin-Laskowsky was carried out. The barium was carefully removed with sulfuric acid; a slight excess of ammonia was added to the filtrate which was evaporated. The dry residue was taken up in strong ammonia. The solution was placed in a sealed tube and heated to 134°C. for six hours as recommended by Scheibe (15). After cooling the solution was taken from the tube and left standing in the air, whereupon a blue color developed. The reaction was positive. Henkel says that this reaction is given by citric and aconitic acids but the latter does not yield pentabromacetone under the conditions of our experiments.

[1] The impurities included in such specimens may also give rise to carbon monoxid, and account for the discrepancy between results of the two methods. For instance, a specimen dried at 164°C. weighing 141.5 mgm. gave pentabromacetone corresponding to 54 mgm. citric acid or 101.7 mgm. of dry barium citrate. The carbon monoxid method indicated 87 mgm. citric acid in 159.2 mgm. of this specimen, while the calculation, from the pentabromacetone determination gave 60.8 mgm. of citric acid. The mixture became black when some of the substance was heated with sulfuric acid.

[2] The fact that this precipitate formed at acid reaction made us think of dicalcium citrate as obtained by Henkel (8) from milk, but the specimen dried at 100°C. gave 27.2 per cent calcium oxid on burning; dicalcium citrate yields 24.4 per cent, and tricalcium citrate 32.55 per cent.

Several substances such as aconitic, itaconic and citraconic acids yield a positive Denigès' reaction, although they do not behave exactly as citric acid does in the course of the reaction. It is not certain that precipitates from these acids contain acetonedicarboxylic acid, particularly, since they do not yield pentabromacetone. Nevertheless, precipitates obtained from our substance by the method of Denigès, treated with hydrogen sulfid, and extracted with ether, on evaporation of the ether left a dry residue which in water solution gave a purple color with ferric chlorid and a positive Legal reaction for acetone. The ferric chlorid reaction could not be obtained if the watery solution had been boiled first; by boiling, acetonedicarboxylic acid is decomposed to carbon dioxid and acetone. Some efforts were made to isolate pure acetonedicarboxylic acid from such precipitates. Denigès reported that it is possible to obtain pure acetonedicarboxylic acid from citric acid by way of the mercury compound. The proof of the purity of this acetonedicarboxylic acid rested on its mercury content.[3] A mercury compound made with acetonedicarboxylic acid prepared by the method of Pechmann contained the same amount of mercury. The formula as signed by Denigès to the mercury compound of acetonedicarboxylic acid is:



It is very doubtful whether the mercury compound of acetonedicarboxylic acid is of uniform composition. Salant and Wise (14) found it necessary to construct a table based on analytical results which gives the weight of the mercury compound obtained from different amounts of sodium citrate under definite conditions. With the aid of this table and within its limits, quantitative determinations of citric acid in aqueous solutions could be made. On repeating the experiment of Denigès we were able to obtain crystalline residues from the ether extract which gave the reactions described by Denigès, but the melting point of these residues varied from about 117 to 125°C. and were associated with decomposition. Denigès did not give the melting

[3] These precipitates when dissolved in dilute hydrochloric acid, and shaken with ether give up to the ether a mercury compound. This property may perhaps be used for purification.

point of acetonedicarboxylic acid prepared by him, and Pechmann (13) gives for the pure acid a melting point of 135°C. with decomposition.[4]

The mercury compound obtained from our substance gave precipitates the consistency and color of which varied from yellow and waxy to white and crystalline. Some of the crystalline precipitates contained white needles, and the melting point varied from 117 to 120°C. with decomposition. There was a great similarity between the properties of the ether residues obtained from our substance and those obtained from citric acid.

The work of Amberg and McClure (2) was undertaken in order to study the fate of citric acid introduced into the organism under various conditions. It was expected that some information would be gained concerning oxidative processes and their disturbances in the organism, but it was not expected that information concerning the oxidative power of organisms in general would be obtained. Dakin (4) very justly says, "The specific character of animal oxidations is most remarkable especially when phenomena such as those presented by diabetes and alcaptonuria are concerned. In these conditions oxidation of a readily oxidizable product of metabolism (glucose, homogentisic acid) may be completely restrained without impairing in the least the capacity of the body for effecting the oxidation of other substances." Batelli and Stern (3) working with animal tissues have shown that the oxidation of various organic acids proceeds under various conditions. Dakin's very interesting review of physiologic oxidations (5) makes the conclusion nearly unavoidable that the organism can bring about oxidations by various mechanisms. The excretion of readily oxidizable substances is of great significance in the light of such considerations, whether these are introduced from without or formed in the organism as an intermediary product of metabolism. The total amount excreted is of importance but of no less importance are the variations in the excretion of the individual acids. It may be suggested that a search for alcohols and aldehydes should be productive of results in certain conditions.

When salts of the lower aliphatic acids are present in sufficient concentrations they may exercise an influence on numerous biologic reactions. Among these are chemotaxis (Wolf, 17), phagocytosis (McJunkin, 12), and enzyme action (Amberg and Loevenhart, 1). Jobling, Eggstein and Petersen (19) showed that citrates particularly have an accelerating influence on tissue and serum esterase.

[4] Crude acetonedicarboxylic acid is readily prepared by the method of Pechmann, by the modification by Koessler and Hankel (10), or by the method of Denigès, but the purification is rather difficult.

In such reactions the individual acid exercises a more or less specific function:

In summarizing it may be said that the substance isolated from urine in the form of an impure barium compound had the following properties in common with citric acid.

1. It gave the pentabromacetone and the typical Denigès reactions.
2. It yielded pentabromacetone and carbon monoxid in amounts closely equivalent.
3. It did not char on heating with concentrated sulfuric acid.
4. It gave the Sabanin-Laskowsky reaction.
5. Precipitates obtained according to Denigès' method behaved very similarly to precipitates from citric acid. From these precipitates a dry ether residue could be obtained, the aqueous solution of which gave the characteristic color reaction for acetonedicarboxylic acid with ferric chlorid.

The results support our contention that citric acid occurs in the urine of normal human beings, but still, they do not convey absolute proof, which rests solely on the isolation of the acid or its salts in a form pure enough for identification.

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THE ADAPTATION OF THE PENTABROMOACETONE METHOD TO THE QUANTITATIVE DETERMINATION OF CITRIC ACID IN THE URINE.*

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The determination of citric acid by the pentabromoacetone method[1] depends upon the formation of water-insoluble pentabromoacetone, when citric acid is oxidized by potassium permanganate in the presence of bromine. The quantitative results would, of course, be vitiated by any impurity of the pentabromoacetone.

When McClure and Sauer[2] employed in urine Kunz's[3] modification of the pentabromoacetone method for citric acid determination, they, at times, found a considerable residue upon treating the pentabromoacetone precipitate with acetone, whereas pure pentabromoacetone is readily dissolved in that solvent. They also found a considerable difference, in many cases, between results obtained by the pentabromoacetone method and those obtained by the Salant and Wise[4] method. These findings pointed out the desirability of further study of the pentabromoacetone method as applied to urine, to ascertain factors interfering with its accuracy.

* The early part of the work was done in conjunction with Dr. Hugh Macdonald.

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[4] Salant, W., and Wise, L. E., The action of sodium citrate and its decomposition in the body, *J. Biol. Chem.*, 1916–17, xxviii, 27.

The following experiment suggested a possible source of error: Urine was treated with sulfuric acid and an excess of bromine, shaken, and allowed to stand. This produced a precipitate, containing bromine, which was only partially soluble in alcohol, ether, or acetone. The alcohol, ether, and acetone solutions of this bromine precipitate were red-brown in color and became turbid upon addition of water.

Since, during the test for citric acid in the urine by the pentabromoacetone method, bromine is liberated and sulfuric acid has been added previously, the conditions are present for the formation of the bromine precipitate which was described in the preceding paragraph. Should this precipitate be produced, at least a part of it would be present as an impurity in the pentabromoacetone precipitate and would to that extent vitiate the results of the citric acid determination.

In order to free the pentabromoacetone method, as applied to urine, from an error which might be due to the appearance of this bromine precipitate, the following procedures were tried: (1) Separation of the pentabromoacetone from the bromine precipitate; (2) reduction of the amount of bromine precipitate formed; and (3) a combination of these two procedures.

Separation of the Pentabromoacetone from the Bromine Precipitate.

(*a*) Numerous trials failed to disclose a *solvent* which would serve to separate the bromine precipitate in its entirety from the pentabromoacetone precipitate. (*b*) Pentabromoacetone is readily volatilized by heat. The effect on bromine precipitate of a degree of heat sufficient to volatilize actively pentabromoacetone was tested out. To accomplish this, known quantities of bromine precipitate and pentabromoacetone, each in a porcelain crucible, were heated simultaneously in the electric oven at temperatures approximately 100–105°C. and the loss was determined in each case. Table I shows the results of four such experiments, each substance was charted separately. The heating was prolonged in Experiment 4 for the purpose of more rigorously testing the resistance of the bromine precipitate to this degree of heat.

It is seen from Table I that less than 18 per cent of the bromine precipitate was lost when the temperature was maintained at approximately 100–105°C., while the pentabromoacetone was

practically all driven off under similar conditions. However, in one experiment, which is not included in Table I, 10.1 mg. of bromine precipitate, without a pentabromoacetone control, were heated at approximately 100°C. for 24 hours with a loss of 2.5 mg.

TABLE I.

Results of Heating Simultaneously Bromine Precipitate and Pentabromoacetone.

Experiment No.	Bromine precipitate used.	Heated.	Temperature (approximately).	Loss.	Percentage lost.
		Results of heating bromine precipitate.			
	mg.	*hrs.*	*°C.*	*mg.*	*per cent*
1	90.5	4½	100	9.5	10.5
2	73.8	5½	105	9.1	12.3
3	86.5	8½	100	10.9	12.6
4	16.8	39½	105	2.9	17.3
		Results of heating pentabromoacetone.			
1	88.6	4½	100	88.2	99.5
2	69.0	5½	105	68.9	99.9
3	70.6	8½	100	70.6	100.0
4	163.2	39½	105	162.9	99.8

TABLE II.

Results of Heating Mixtures of Bromine Precipitate and Pentabromoacetone.

Mixtures.	Used.	Heated.	Temperature (approximately).	Loss.	Remaining.
	mg.	*hrs.*	*°C.*	*mg.*	*mg.*
Pentabromoacetone	52.0	9½	100	51.9	1.2
Bromine precipitate	1.1				
Pentabromoacetone	50.3	24	100	51.8	8.7
Bromine precipitate	10.2				

or 24.8 per cent. This percentage of loss was unusually high and was encountered only in this one experiment.

Next, mixtures of small quantities of bromine precipitate and relatively large quantities of pentabromoacetone were heated. Table II shows the results of two such experiments.

These results are such as would be expected after consideration of the findings presented in Table I. In the first experiment the loss upon heating the mixtures was about equal to the quantity of pentabromoacetone employed. In the second experiment, where a larger quantity of bromine precipitate was used and longer heating was carried out, the loss was somewhat greater than the amount of pentabromoacetone. This latter finding is readily explainable on the basis of a partial volatilization of the bromine precipitate.

Also determinations of pentabromoacetone were made in duplicate, in two specimens of native urine with heating used as an additional step to the pentabromoacetone method. Table III gives the results of these determinations.

TABLE III.

Pentabromoacetone Obtained from Urine, Using Heat as an Additional Step in the Pentabromoacetone Method.

Normal urine, 50 cc.	Pentabromoacetone found.	Residue after heating.	Heated.	Temperature.
	mg.	*mg.*	*hrs.*	*°C.*
Mixed specimen from adults.	12.3 12.6	2.9 2.9	22½	100–105
Mixed specimen from children.	30.5 29.7	6.4 7.8	18½	100–106

Reduction of the Amount of Bromine Precipitate.

It was found that a preliminary treatment of the urine with charcoal[6] causes a marked diminution of the quantity of the bromine precipitate which is produced when urine is treated with sulfuric acid and an excess of bromine. This was true, whether the urine was boiled with sulfuric acid and charcoal, or whether the unheated urine was rendered alkaline by sodium hydroxide and only shaken with charcoal. This was demonstrated in the following way: From 150 cc. quantities of a specimen of urine, 45 mg. of bromine precipitate were obtained when charcoal was not used, and 3.9 mg. of bromine precipitate when the urine had been previously treated by boiling with sulfuric acid and charcoal

[6] Blutkohle, Kahlbaum, was used in all the experiments.

and filtered. A different specimen of urine yielded 31.3 mg. of bromine precipitate per 150 cc. without charcoal treatment, and only 0.1 mg. of bromine precipitate after being rendered alkaline with sodium hydroxide and shaken, unheated with charcoal.

Amberg and McClure[6] had found that to boil urine with sulfuric acid and charcoal may cause a considerable loss of citric acid—as much as 27 per cent in a solution containing the equivalent of 30 mg. of citric acid, in the form of its sodium salt, in 50 cc. of water. It was therefore evident that this method of using charcoal was unavailable for our purpose.

However, it was found in this study that the use of charcoal in an unheated alkaline solution caused little or no loss of citric acid. For example, by using 50 cc. quantities in duplicate, from an aqueous solution of sodium citrate containing the equivalent of 50 mg. of citric acid in each 50 cc., 51.3 and 51.0 mg. of citric acid,[7] respectively, were determined. This method of employing charcoal, therefore, serves the purpose quite satisfactorily.

Combination of Reduction in the Formation of Bromine Precipitate and Separation of Pentabromoacetone from the Bromine Precipitate.

In view of the results reported above, it seemed that a combination of a preliminary treatment of the urine by rendering it alkaline, and shaking with charcoal without heating, and of separation of the pentabromoacetone from impurities by volatilization might be advantageous. To test this out, the pentabromoacetone method for citric acid determination with these two steps added[8]

[6] Amberg, S., and McClure, W. B., The occurrence of citric acid in urine, *Am. J. Physiol.*, 1917, xliv, 453.

[7] Throughout this work in computing the amount of citric acid, 5 mg. were added for each 50 cc. of solution tested, because of an undetermined rest which Amberg and McClure[6] had found to exist.

[8] The pentabromoacetone method for citric acid in urine, as modified, is: Approximately 200 cc. of urine rendered alkaline to litmus with 5 per cent NaOH are filtered. To 150 cc. of filtrate 3 gm. of charcoal are added and the mixture is shaken vigorously unheated for 1 minute and filtered. Duplicate specimens of 50 cc. of the filtrate are taken and to each, 1 cc. of dilute H_2SO_4 is added. Bromine vapor is poured in. If the solution remains clear, 10 cc. of 1:1 H_2SO_4 and 3 cc. of 37.5 per cent KBr are added and the solution is heated in a water bath at 50–55°C. for 5 minutes. 20 cc. of 5

was applied to specimens of urine to which known quantities of sodium citrate had been added. Table IV gives the results of these experiments.

TABLE IV.

Results of Duplicate Determinations of Citric Acid, in Urine by the Pentabromoacetone Method, Using Preliminary Charcoal Treatment and Separation of the Pentabromoacetone from Impurities in the Final Precipitate by Heat.*

Experiment No.	Citric acid.				
	Added.	Recovered.	In native urine (same method).	Net recovered.	Recovered.
	mg.	*mg.*	*mg.*	*mg.*	*per cent*
1	10.0	21.6	10.3	Average 10.7	107.0
	10.0	21.4	11.2		
2	20.0	30.1	10.4	Average 19.6	98.0
	20.0	30.6	11.2		
3	30.0	43.3	13.8	Average 29.9	99.7
	30.0	44.3	14.0		
4	40.0	50.0	12.6	37.4	93.5
	40.0	50.7	10.7	40.0	100.0
5	40.0	53.3	13.2	Average 38.95	97.0
	40.0	51.1	13.3		
6	40.0	54.9	17.0	37.9	94.8
	40.0	56.3	17.0	39.3	98.0
7	50.0	63.3	13.8	Average 48.9	97.8
	50.0	62.4	14.1		

* Throughout the experiments 50 cc. quantities of the solutions being tested for citric acid, were used for each determination and bromine vapor was added instead of bromine water, following the suggestion of Amberg and McClure.[4]

per cent $KMnO_4$ solution are added drop by drop with vigorous shaking. Concentrated $FeSO_4$, acidified with H_2SO_4, is added, slowly with shaking, in amount sufficient to remove the undissolved $MnO_2 \cdot H_2O$ and excess of bromine. The precipitate is weighed in a Gooch crucible, after drying 24 hours in a desiccator. The crucible and contents are now heated over night in the electric oven at 100–105°C. (A much shorter period of heating is usually sufficient but necessitates reheating and an additional weighing to determine whether all the pentabromoacetone has been volatilized off.) After cooling the crucible is again weighed and the loss of weight due to heating is taken as equivalent to the quantity of pentabromoacetone. From the pentabromoacetone, the quantity of citric acid $+ 1H_2O$ is calculated by multiplying by 0.464 and adding 5 mg., because of an undeterminable fraction.

CONCLUSIONS.

The pentabromoacetone method may be used for the quantitative determination of citric acid in normal urine with satisfactory results, if the unheated urine has been rendered alkaline, previously, by sodium hydroxide, and shaken with charcoal and filtered; and if the final precipitate is heated as a means of separating pentabromoacetone from impurities.

This work was undertaken at the suggestion of Dr. Samuel Amberg to whom I am indebted for much helpful advice.

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COMPARISON OF PENTABROMACETONE METHOD, AND SALANT AND WISE'S METHOD FOR CITRIC ACID DETERMINATION IN URINE

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Applying the "pentabromacetone method"[1] for citric acid determination (1), Amberg and McClure (2) were the first to note the presence of citric acid in the urine of normal children and adults. They also obtained positive tests for citric acid in normal urine with the method of Salant and Wise[2] (3), which is based upon Denigès' reaction.

[1] Briefly the pentabromacetone method, as we applied it, is: To 50 cc. of filtered urine, 1 cc. of dilute H_2SO_4 is added and bromine vapor poured in. The clear solution, to which KBr and strong H_2SO_4 are added, is heated in a water bath at 50 to 55°C. for 5 minutes, after which 5 per cent $KMnO_4$ solution is added slowly. $MnO_2 \cdot H_2O$ and the excess bromine are removed by addition of $FeSO_4$ acidified with H_2SO_4. The precipitate obtained is weighed in a Gooch crucible, after drying 24 hours in a desiccator. It is then washed with acetone which dissolves out the pentabromacetone very quickly. After this, the crucible is again dried and weighed. From the pentabromacetone, we calculate the quantity of citric acid present, adding 5 mgm. in each case, because of an undeterminable fraction, which Amberg and McClure (2) had found to exist.

At times the acetone-insoluble part of the precipitate was considerable, as much as 9.8 mgm. Thus far the nature of this residue has not been determined.

[2] Salant and Wise's method, as applied by us is as follows: From a specimen of urine, which if not already acid to litmus is made so by addition of HCl, three portions of 10, 15 or 20 cc. each (quantity selected so that weight of Denigès' precipitate fell well within the limits of the table of Salant and Wise) were taken. These portions were evaporated nearly to dryness over water bath; volume of each was made up with water to 5 cc.; sufficient 10 per cent barium acetate added to insure excess of barium ion. Amount of barium acetate required was determined by a test on one of the three portions which was then discarded. Remaining two portions were made faintly alkaline with Ba $(OH)_2$; three volumes of 95 per cent alcohol were added; the mixtures allowed to stand an hour; centrifuged and the supernatant liquid poured off. The sediment was twice washed by adding 30 to 40 cc. of 50 per cent alcohol, stirring, centrifuging and discarding the supernatant fluid. The precipitate was treated with 10 drops of syrupy H_3PO_4

For the purpose of comparing the accuracy and practicability of these two methods for quantitative work in urine, we applied them to the determination of citric acid:

I. In normal urine
   a. Specimens from a series of children of various ages.
   b. Specimens from the same child on successive days.
II. In normal urine to which known quantities of citric acid, in the form of sodium citrate, had been added.

The results of the experiments for the comparison of the two methods applied to normal urine from a series of children are tabulated, as shown in table 1, *a*.

These children were chiefly orthopedic patients who were afebrile at the time when the urine was collected. The figures give some idea of the 24-hour excretion of citric acid during childhood. It is difficult to obtain 24-hour collections of urine from young children and, as Amberg and McClure (2) stated, the pentabromacetone method for quantitative determination of citric acid in urine is not yet as accurate as it might be.[3] For these reasons the figures given can only be regarded as approximate. The results obtained by the two methods show poor agreement in a considerable number of cases.

The data of the experiments for the comparison of the two methods when applied to the 24-hour specimens of normal urine, collected on successive days, are shown in table 1, *b*.

and transferred to a test tube by a jet of hot water. Residual alcohol was driven off by boiling and volume brought to approximately 10 cc. by evaporation. After addition of 0.7 cc. of Denigès' reagent, the mixture was heated to boiling; cooled; volume made up to 12 cc. and filtred. To one-half of filtrate, 0.35 cc. of Denigès' reagent was added and the volume brought to 6 cc. Solution was heated to boiling and 2 per cent $KMnO_4$ added drop by drop until a persistent pink or deep brown color appeared. Mixture was decolorized by three drops of H_2O_2, heated to boiling, cooled, and filtered on a weighed Gooch crucible. The precipitate was washed with water, then alcohol, and finally ether, as directed by Salant and Wise; dried at 90 to 100°C., and weighed. Using the equivalent of sodium citrate, as obtained from Salant and Wise's table, we computed the 24-hour excretion of citric acid.

The figures for citric acid, shown in the tables, for both the pentabromacetone method and Salant and Wise's method represent the average of duplicate determinations.

[3] An improvement of the pentabromacetone method, developed subsequently, will be published elsewhere by McClure.

TABLE 1, *a*

Quantities of citric acid found by the two methods in urine from children of various ages

NUMBER	AGE	URINE IN 24 HOURS	SPECIFIC GRAVITY	CITRIC ACID EXCRETED IN 24 HOURS	
				Pentabromacetone method	Salant and Wise's method
		cc.		*mgm.*	*mgm.*
1	7 months	320	1008	44	52
2	9 months	365	1009	96	80
3	14 months	594	1008	206	253
4	14 months	325	1007	53	52
5	15 months	380	1009	51	73
6	17 months	630	1007	134	130
7	25 months	222	1027	76	115
8	27 months	225	1032	213	180
9	33 months	190	1026	49	78
10	3¼ years	375	1019	99	91
11	3¾ years	176	1026	64	51
12	3 7/12 years	304	1021	149	146
13	4 1/12 years	265	1033	131	139
14	4¾ years	490	1014	102	150
15	4¾ years	375	1026	132	145
16	4¾ years	350	1030	254	152
17	5⅔ years	250	1030	127	121
18	5 1 1/2 years	227	1022	30	37
19	5 1 1/2 years	425	1019	134	102
20	6 1/12 years	850	1011	112	238
21	7 years	425	1015	182	167
22	7 2/12 years	550		79	98
23	7½ years	300	1025	112	84
24	7¾ years	450	1018	209	143
25	8¼ years	500	1020	296	294
26	8⅓ years	460	1021	230	247
27	8½ years	550	1016	154	165
28	8 1 0/12 years	425		82	92
29	9 years	650	1009	124	140
30	9½ years	890	1008	192	198
31	9½ years	795	1011	239	231
32	9½ years	740	1021	431	258
33	10 2/12 years	720	1017	269	259
34	10½ years	305	1026	88	88
35	10 5/11 years	560	1015	114	139
36	11⅓ years	275	1033	257	190
37	12 years	522	1019	283	156
38	13 years	510	1024	240	187
39	14 years	1005	1017	241	177

TABLE 1, *b*

Quantity of citric acid from 24-hour collections of urine of a child aged 6 years and 2 months, collected during two periods of 3 days each

DATE	URINE IN 24 HOURS	SPECIFIC GRAVITY	PENTABROMACETONE METHOD	SALANT AND WISE'S METHOD
	cc.		*mgm.* citric acid	*mgm.* citric acid
4/18/18	615	1016	241.0	196.0
4/19/18	1100	1008	239.8	234.4
4/20/18	400	1016	135.2	124.0
4/24/18	480	1011	128.6	120.0
4/25/18	910	1006	200.2	212.0
4/26/18	750	1009	210.0	236.0

The excretion of citric acid was fairly uniform on 4 days. During 2 days the quantity of urine collected was low, as was also the amount of citric acid estimated.

For the purpose of controlling the accuracy of the two methods, we added known amounts of sodium citrate to normal urine and determined what amounts of citric acid could be recovered. The results are set forth in the following table:

TABLE 2

Quantity of citric acid from normal urine to which known amounts of sodium citrate had been added. Table gives milligrams of citric acid per 50 cc. of urine

NUMBER	PENTABROMACETONE METHOD				SALANT AND WISE'S METHOD			
	Native urine	Native urine plus 13.6 mgm. citric acid (as sodium salt)	Recovered		Native urine	Native urine plus 13.6 mgm. citric acid (as sodium salt)	Recovered	
			Amount	Per cent			Amount	Per cent
1	18.7	30.0	11.3	83	15.5	21.0	5.5	40
2	13.4	26.2	12.8	94	15.3	22.5	7.2	53
3	12.8	24.7	11.9	88	14.3	26.5	12.2	90
4	20.6	33.1	12.5	92	15.1	25.1	10.0	74

It is seen that in all experiments, except the third, a larger percentage of the added citrate is determined by the pentabromacetone method.

SUMMARY

Comparison of the results obtained by the two methods for the determination of citric acid in urine shows: 1st, that out of 39 cases, reported in table 1, *a*, there was fair agreement in 15 cases; 2nd, that there was better agreement in the case reported in table 1, *b*.

In the control experiments reported in table 2, where known quantities of sodium citrate were added to normal urine, the pentabromacetone method gave much better results than Salant and Wise's method in 3 cases. In the fourth case the results by the two methods showed close comparison.

It would seem that, for quantitative work in urine, the pentabromacetone method of citric acid determination is preferable.

We wish to acknowledge our indebtedness to Dr. Samuel Amberg for his advice and counsel throughout this work.

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THE PRESENCE OF FORMIC ACID IN THE URINE OF INFANTS AND OLDER CHILDREN *

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Formic acid has been found in the urine of normal adults by several investigators.[1] With the intention of studying its presence in the urine of children, we examined fifty-six specimens from ten infants and nine older children for a period, in most cases, of three days each. The patients were suffering from various diseases (Tables 1 and 2). The urine in all cases was negative to copper reduction tests.

The method of Autenrieth for the determination of formic acid in the urine was used. Three hundred c.c., or less, of urine, was distilled over a free flame, after the addition of 30 c.c. of 25 per cent. phosphoric acid, until about 75 c.c remained in the flask, which was then filled to the original volume by water from a separatory funnel. The distillation was continued until the distillate was no longer acid to litmus (about 1,200 c.c. in our experiments). The distillate was then evaporated to a small volume and treated with mercuric chlorid. Formic acid, by virtue of its strong reducing power, converts mercuric chlorid to mercurous chlorid, which is filtered on a Gooch crucible and weighed. Each gram of mercurous chlorid obtained represents 0.0977 gm. formic acid. Autenrieth found that the average twenty-four hour output of formic acid by a normal adult is 0.28 gm. Another normal adult excreted 0.091 gm. in twenty-four hours. We used this method because of our inability, for several months, to secure suitable apparatus for ether

* The investigation was carried on in the Otho S. A. Sprague Memorial Institute Laboratory of the Children's Memorial Hospital, Chicago, and in the Section on Pediatrics of the Mayo Clinic under the direction of H. S. Amberg, Rochester, Minn.

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TABLE 1.—FORMIC ACID IN THE URINE OF INFANTS

Case	Age, Mos.	Diagnosis	Date, 1920	Weight Lb.	Weight Oz.	Urine, C.c.	Sp. Gr.	Formic Acid Mg.	Formic Acid Average for 3 Days, Mg.	Diet	Stools	Remarks
751 P. P.	4	Gastro-enteritis	9/26	8	14	270		6.67		Whole milk 10 oz.; Boiled water 10 oz.; Dextrose maltose 1 oz. — 5 oz. × 4		Convalescent intoxication; received injections of glucose acacia
			9/27	8	14	285	1.004	5.02				
			9/28	8	10	205		3.65	5.11			
										Albumin milk (Hoos) 9 oz.; Dextrose maltose 0.25 oz. — 4.5 oz. × 2; Cereal 1 oz. × 1		
751 P. P.	5	Gastro-enteritis	10/ 2	9	0	190		3.33		Whole milk 16 oz.; Boiled water 11.5 oz.; Dextrose maltose 1 oz. — 5.5 oz. × 3; Cereal 1 oz. × 2	Normal 1 to 2 daily	Convalescent intoxication; infection originating in the ears; died 10/27/20
			10/ 3	8	13	210		5.31				
			10/ 4	8	14½	210	1.007	7.29				
			10/ 8	8	12	200	1.006	5.11	5.31			
289 J. G.	5	Gastro-colitis	9/29	8	7	285	1.008	11.46		Albumin milk (Hoos) 12 oz.; Boiled water 9 oz.; Dextrose maltose	Normal 1 to 2 daily	Moderately severe entero-colitis; improved, discharged
			9/30	8	5	265	1.008	8.59				
			10/ 1	8	5	125	1.011	2.71				
			10/ 6	8	4	135	1.006		7.58			
261 H. J.	6	Atrophy	10/12	8	1½	310	1.006	13.99		Albumin milk (Hoos) 24 oz.; Dextrose maltose 1 oz. — 4 oz. × 6	Normal 1 in 2 to 3 days	Marasmus; improved, discharged
			10/13	8	½	370	1.008	16.62				
			10/14	8	4	230	1.007	8.03	12.54			
261 H. J.	6½	Atrophy	10/25	8	0	295	1.010	25.13		Whole milk 15 oz.; Water 10 oz.; Farina 1 oz.; Dextrose maltose 1 oz. — 4 oz. × 6	Normal 1 daily	
			10/26	8	0	300	1.008	5.04				
			10/27	8	8	295	1.006	8.63	12.60			
235 C. L.	2	Entero-colitis	10/12	6	8	240	1.006	6.73		Albumin milk (Hoos) 18 oz.; Boiled water 6 oz.; Dextrose maltose 1 oz. — 4 oz. × 6	Loose 1 daily	Mild diarrhea; improved, discharged
			10/13	6	8½	240	1.004	2.42				
			10/14	6	9	375	1.005	6.51	5.22			
246 C. V.	8	Entero-colitis	10/15	7	15	335	1.008	9.03		Whole milk 10 oz.; Boiled water 10 oz.; Dextrose maltose 1 oz.; Maltose soup 0.5 oz. — 4 oz. × 5	Normal 1 to 2 daily	Probably infantilism; improved, discharged; gained well
			10/17	8	1½	430	1.006	7.31				
			10/18	8	½	280	1.007	7.60	8.31			
268 J. G.	7½	Atrophy	10/18	7	10½	370	1.006	6.34		Whole milk 15 oz.; Water 12 oz.; Farina 1 oz.; Dextrose maltose 1 oz. — 4.5 oz. × 6	Normal 1 to 3 daily	Marasmus; improved, discharged
			10/19	7	13½	360	1.005	21.06				
			10/20	7	10½	205	1.008	10.45	12.61			
754 W. G.	8	Gastro-enteritis	10/25	11	7½	360	1.008	9.07		Whole milk 12 oz.; Boiled water 12 oz.; Dextrose maltose 1 oz. — 4 oz. × 6; Cereal 0.5 oz. × 6	Normal constipated	Dyspepsia; improved, discharged
			10/26	11	5	260	1.006	7.38	8.22			
240 R. K.	2	Entero-colitis	10/30	8	3	220	1.010	8.44		Albumin milk (Hoos) 24 oz.; Dextrose maltose 1 oz.	Loose 2 to 3 daily	Improved, discharged
			10/31	8	3½	195	1.012	3.01	3.22			
188 R. B.	5	Otitis media	10/19	14	0	140	1.010	7.12		Whole milk 18 oz.; Boiled water 12 oz.; Dextrose maltose 1 oz. — 6 oz. × 5	Normal 1 to 2 daily	Severe enteritis and meningitis; died 10/31/20
			10/20	14	0	340	1.007	21.28				
			10/22	14	0	190	1.010	21.20	16.50			

TABLE 2.—Formic Acid in the Urine of Children

Case	Age, Years	Diagnosis	Date	Weight, Pounds	Urine, C.c.	Specific Gravity	Formic Acid		Diet	Remarks
							Mg.	Average for Three Days, Mg.		
243 F. G.	7 5	Chorea	11/ 3 20 11/ 7/20 11/ 8 20	45.5	235 845 840	1.030 1.018 1.012	6.83 19.37 6.15	10.78	General, soft	Patient improved, in bed; treatment for chorea
710 A. S.	4.5	Lobar pneumonia	11/ 3/20	34	325	1.026	5.38		General	Convalescent pneumonia; patient up and about in the ward
191 L. E.	2.25	Otitis media	11/17 20 11/18/20	25	460 390	1.030 1.022	4.41 10.09	7.25	General	Up and about ward
172 T. J	7	Cervical adenitis	11/ 8 20	50	820	1.023	19.32		General	Tonsils and adenoids removed; condition good
2544 M B	5	Enlarged tonsils and adenoids	11/ 9 20 11/10 20 11/11/20	47	810 790 1025	1.012 1.019 1.015	4.44 12.59 6.66	7.89	General	Chorea improved, patient in bed
245 R. P.	8.5	Chorea	11/12/20 11 13/20 11/14/20	48	970 500 840	1.014 1.018 1.012	11.95 14.81 12.7	13.15	General	Chorea improved; patient in bed
246 G. W.	9	Chorea	11/15/20 11/16 20 11/17/20	58	275 700 450	1.010 1.012 1.012	6.63 9.22 8.01	7.95	General	Chorea moderately severe; patient in bed
242 E. M.	10.75	Chorea	11/ 9 20 11 10/20 11 11/20	65	570 790 1045	1.030 1.019 1.016	22.80 31.03 39.43	31.08	General	Improved; up and about ward
215 T. H.	10.5	Chorea	11/12 21 11 13/21 11/14/21	57	750 700 880	1.020 1.020 1.020	11.82 15.62 19.39	15.61	General	Chorea improved; patient in bed

extraction of fluids. When, later, control experiments were made with the Lind apparatus, our results indicated the presence of formic acid in the urines analyzed by the former method. Dakin, Janney and Wakeman, working with the ether extraction method, found the average amount of formic acid in the urine of adults to be only 60 mg., with variations from 29.9 to 118.6 mg.

Any method involving distillation with acids is open to objection because formic acid may be derived from glucose and, perhaps, from other substances. There is no doubt that during the distillation some formic acid can arise from this source, as was shown by an increase

TABLE 3.—Formic Acid Determinations

Experiment	Autenrieth Method, Mg. for Each 100 C.c.	Ether Extraction Method, Mg. for Each 100 C.c.
1	3.27	3.40
2	2.37	2.17
3	3.62	3.96
4	2.73	2.81
5	4.93	3.93
6	2.16	2.15

in the formic acid determinations of a specimen of urine from 21.7 to 106 mg. after the addition of 2 per cent. glucose. On the other hand, such a formation of formic acid apparently need not always occur, as is shown by the results obtained with parallel determinations of formic acid by the acid distillation method of Autenrieth and the ether extraction method (Table 3).

While the Autenrieth method is open to objections, the results obtained by parallel determination with the ether extraction method have been so close that we can safely conclude that the urine of children does in all likelihood contain formic acid.

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THE BACTERIOLOGY OF THE NORMAL INFANT'S URINE *

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It has been rather generally accepted that the urine of normal infants often contains a small number of organisms that cannot definitely be excluded as contaminations. The work of Ross,[1] Beeler and Helmholz,[2] Kleinschmidt,[3] and others substantiates this view. Recently the entire question was reopened by Langer and Soldin [4] who claim that all former work in which liquid mediums had not been used for the isolation of bacteria is open to criticism. In a series of 138 cases they isolated *Streptococcus lacticus* 128 times, *Bacillus coli* sixteen times, and *Bacillus lactis aerogenes* four times; *Bacillus coli* was associated with *Streptococcus lacticus* six times. Occasionally, a single examination revealed sterile urine, but on repetition bacteria were found. At that no account is taken of anaerobic bacteria.

Langer and Soldin speak only of washing the urethra thoroughly with a 10 per cent. boric acid solution. The solvent for the boric acid is not mentioned, and in a single sentence, without any citation of experiments, they consider all portions of the urine alike with regard to bacterial content and formed elements. The most striking features of their series are the constant presence of bacteria of the colon group and the complete absence of staphylococci which are normally present in the female urethra. This is in direct contrast to the findings of Beeler and Helmholz,[2] namely, that only exceptionally were bacilli of the colon group found, and when organisms were present they were usually staphylococci or diphtheroids. Kleinschmidt cultured, on solid

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mediums, fifty-four specimens of urine from female patients and obtained sterile urine in all but ten, while in liquid mediums he obtained bacteria in thirty-two of sixty-two cultures. Further, he says that he was rarely able to obtain the same flora in successive examinations, which he interprets as the result of varying contaminations. Dextrose bouillon yielded positive cultures more often than plain bouillon; the percentages, however, are not indicated. He isolated *Streptococcus lacticus* more often than any other organism; second in line are members of the colon group, third the staphylococci.

Inasmuch as the former work of one of us was carried on entirely on various solid mediums, it was thought wise to repeat the work, using also the liquid culture medium suggested by Langer and Soldin. This

TABLE 1.—Results of Cultures

No.	Sex	Specimen 1		Specimen 2	
		Liquid Medium	Solid Medium	Liquid Medium	Solid Medium
19	Boy	Bacillus subtilis	Negative	Negative	Negative
28	Girl	Negative	Negative	Staphylococcus	Negative
30	Boy	Negative	Negative	Gram-positive bacillus spore-former	Negative
31	Girl	Gram-negative streptococcus not identified	Negative	Negative	Negative
40	Boy	Gram-positive spore-former	Negative	Negative	Negative
80	Girl	Gram-positive diplococcus not Streptococcus lacticus	Negative	Negative	Negative
10*	Boy	Gram-positive staphylococcus after one week	Negative		
32*	Girl	Gram-negative bacillus short, Bacillus subtilis	Negative		
33*	Boy	Staphylococcus	Negative		
34*	Girl	Bacillus subtilis	Negative		
77*	Boy	Gram-positive staphylococcus	Negative		

* One specimen only was obtained.

seemed the more important as the constant presence of *Streptococcus lacticus* in the normal urine would be of great importance in settling the problem of the mode of infection in pyelitis.

The infants from whom the urine was taken for this study ranged in age from 6 weeks to 16 months. All had normal temperatures and were normal or nearly normal in weight. All were free from any infection or disease, as indicated by physical examination. Some of the infants were entirely breast fed, others were breast and bottle fed, and still others were fed entirely with artificial food.

The technic of obtaining urine was practically the same as that described by Beeler and Helmholz. The catheters used were of metal, varying from 1.5 to 2.5 mm. in diameter, with a short rubber tube attached by means of which the flow of urine could be controlled and collected in two separate tubes. The catheters were sterilized indi-

vidually in large test tubes so that they could be removed without danger of contamination.

To catheterize a patient three persons were always at hand; one to hold the limbs of the child, one to separate the labia, cleanse the parts, and pass the catheter, and a third to play the stream of compound solution of cresol, pass out the catheter, and hold the test tubes to receive the urine.

The meatus was cleansed as follows: The labia were held apart or the foreskin was retracted and the debris loosened by a small sterile applicator, while a steady stream of 1 per cent. compound solution of

TABLE 2.—Results of Cultures

No.	Sex	Specimen 1			Specimen 2		
		Liquid Medium	Solid Medium	Colonies in Each C.c.	Liquid Medium	Solid Medium	Colonies in Each C.c.
12	Girl	Negative.....	Negative.....	0	Staphylococcus	Staphylococcus	1,000
19	Boy	Bacillus subtilis	Negative.....	0	Negative.....	Negative.....	0
26	Boy	Negative.....	Negative.....	0	Bacillus subtilis	Bacillus subtilis	Countless
28	Girl	Negative.....	Negative.....	0	Staphylococcus	Negative.....	0
30	Boy	Negative.....	Negative.....	0	Gram-positive bacillus, spore-former	Negative.....	0
31	Girl	Streptococcus, not identified	Negative.....	0	Negative.....	Negative.....	0
40	Boy	Gram-positive bacillus, spore-former	Negative.....	0	Negative.....	Negative.....	0
48	Boy	Staphylococcus	Staphylococcus	1	Negative.....	Negative.....	0
78	Boy	Gram-positive staphylococcus and non-pathogenic bacillus	Gram-positive staphylococcus and non-pathogenic bacillus	4	Negative.....	Negative.....	0
80	Girl	Gram-positive diplococcus not S. lacticus	Negative.....	...	Negative.....	Negative.....	0

cresol was played on the region of the meatus. A dry sterile applicator was then applied directly over the meatus to remove any excess fluid. The catheter was then inserted. If there was enough urine in the bladder two specimens were collected.

The urine was cultured in tubes containing 1 per cent. dextrose broth titrated to a hydrogen ion concentration of from 7.6 to 7.8 on plates of litmus-lactose-agar, made by adding 1 per cent. agar, 1 per cent. lactose and 1 per cent. litmus to plain broth that had been titrated to p_H 7.6-7.8. About 1 c.c. of urine was used in about 8 c.c. of broth, and from 0.5 to 1 c.c. for the plating in litmus-lactose-agar. The number of colonies of bacteria indicated in Tables 2 and 3 represent the number of bacteria for each cubic centimeter of urine. All streptococci were subcultured in litmus milk for identification. *Streptococcus*

lacticus was identified by a method very kindly furnished us by Prof. E. G. Hastings of the University of Wisconsin. The identification is based on the fact that *Streptococcus lacticus* reduces the litmus before curdling the milk, and the other streptococci produce a pink curd in the milk without reducing the litmus.

Seventy infants, thirty-five girls and thirty-five boys, were studied. The findings can best be grouped under headings as follows:

1. *All Cultures Negative.*—In this group there were thirty infants, seventeen girls and thirteen boys. In only twenty-one of the thirty was it possible to obtain enough urine for two specimens. This group represents 40 per cent. of the samples examined and in two-thirds of these the sterility of the urine was determined in duplicate on Specimens 1 and 2. The cultures in cases in which two specimens were obtained are included in this group only when all four were sterile. In the others of this series one specimen only was obtained, and cultures were sterile in both liquid and solid mediums.

2. *Cultures in Solid Mediums Negative, in Liquid Mediums Positive.*—Much to our surprise, this group was relatively small, eleven cases (five girls and six boys). Langer and Soldin's work led us to believe that this would make up the largest group of the series. The various organisms in cultured liquid mediums are given in Table 1. The importance of this group is paramount in disproving Langer and Soldin's contention that liquid mediums are essential for this work. In only 15 per cent. has it made a difference, and even here the significance is doubtful.

3. *Cultures in Solid Mediums Positive, in Liquid Mediums Negative.*—Of the three cases in this group (two boys and one girl), contamination was unquestionable in two; in the third were two colonies of streptococci, which may or may not have been due to contamination.

4. *Cultures of One Specimen Positive, and All Cultures from the Other Specimen Negative.*—Specimens 1 and 2 refer to the first and second portions of urine obtained by catheterization. There were ten cases in the group (six boys and four girls, Table 2). Contrary to expectation Specimen 1 was sterile four times and Specimen 2 showed growth six times, indicating that the first portion of the urine voided does not necessarily contain the contaminating bacteria. In only two of the four was there growth in the solid mediums inoculated from Specimen 2.

5. *All Cultures Positive.*—In most of the twenty-six cases in this group (fourteen boys and twelve girls), all the cultures showed growth although occasionally one or another was negative. The num-

TABLE 3.—RESULTS OF CULTURES

No.	Sex	Specimen 1			Specimen 2		
		Liquid Medium	Solid Medium	Colonies in Each C.c.	Liquid Medium	Solid Medium	Colonies in Each C.c.
2	Girl	Negative.....	Negative.....	0	Gram-positive staphylococcus, large	Gram-positive staphylococcus, large	1,000
5	Girl	Gram-positive streptococcus not identified	Gram-positive staphylococcus, large	920			
6	Girl	Gram-positive streptococcus not identified, gram-negative bacillus non-pathogenic	Gram-negative streptococcus not identified, gram-negative bacillus, gram-negative staphylococcus	570			
8	Girl	Gram-negative bacillus probably B. coli, gram positive streptococcus not identified	Gram-negative bacillus probably B. coli	800			
22	Boy	Gram-positive streptococcus and Streptococcus lacticus	Bacillus coli	130	Gram-positive streptococcus and Streptococcus lacticus		
25	Boy	Gram-positive staphylococcus, large	Gram-positive staphylococcus, large	6			
36	Boy	Staphylococcus	.....	..	Staphylococcus	Staphylococcus	38
37	Boy	Staphylococcus, large	Staphylococcus, large	2	Staphylococcus, large	Staphylococcus, large	26
42	Girl	Gram-positive staphylococcus, large, and S. lacticus	Staphylococcus and B. subtilis	20	Gram-positive staphylococcus, large, and S. lacticus	Staphylococcus, B. subtilis, and one colony of S. lacticus	8
44	Girl	.....	.....	...	Streptococcus, not S. lacticus	Streptococcus, not S. lacticus	Few
45	Girl	Streptococcus lacticus and diplococcus	.....	...	Streptococcus lacticus and diplococcus	Streptococcus lacticus and diplococcus	6
47	Girl	Gram-positive staphylococcus	Gram-positive staphylococcus	2	Gram-positive staphylococcus	Negative.....	0
48	Boy	Gram-positive staphylococcus	Gram-positive staphylococcus	1	Negative.....	Negative.....	0
53	Girl	Gram-negative bacillus not B. coli and gram-posiltive staphylococcus	Gram-negative bacillus not B. coli	2			
54	Boy	Gram-positive staphylococcus, large	Staphylococcus, large	58	Few staphylococci, large	Negative.....	0
56	Boy	Gram-negative staphylococcus	Staphylococcus	100	Staphylococcus	Staphylococcus	100
59	Boy	Staphylococcus, large	.....	...	Staphylococcus, large	Staphylococcus, large	50
60	Boy	Gram-positive staphylococcus, large	Staphylococcus, large	6	Streptococcus, not S. lacticus		
61	Boy	Staphylococcus, large	Staphylococcus, large	15	Staphylococcus, large		
64	Girl	Streptococcus lacticus and gram-negative spore-former	Staphylococcus, large and Streptococcus lacticus	3			
67	Boy	Gram-negative staphylococcus	Bacillus subtillis	1			

TABLE 3.—RESULTS OF CULTURES—(*Continued*)

No.	Sex	Specimen 1			Specimen 2		
		Liquid Medium	Solid Medium	Colonies in Each C.c.	Liquid Medium	Solid Medium	Colonies in Each C.c.
74	Girl	Staphylococcus, large and spore-forming bacillus	Staphylococcus, large and spore-forming bacillus	3	Staphylococcus, large		
75	Boy	Staphylococcus, large	Spore forming bacillus	10	Spore-forming bacillus		
76	Boy	Gram-negative bacillus, non-pathogenic	Gram-negative bacillus, non-pathogenic	2	Gram-negative bacillus, non-pathogenic	Negative.....	0
81	Boy	Negative.....	Bacillus coli and Streptococcus lacticus	4	Streptococcus lacticus and Bacillus coli	Streptococcus lacticus and Bacillus coli	200
82	Boy	Negative.....	Staphylococcus, large	1	Gram-negative bacillus, spore-former	Negative.....	0
83	Girl	Staphylococcus, large	Staphylococcus, large	10	Staphylococcus, large, and streptococcus not identified	Staphylococcus, large	6
85	Girl	Two streptococci, one S. lacticus	Negative.....	0	Two streptococci, one S. lacticus	Two streptococci, one S. lacticus	Many

ber of bacteria for each cubic centimeter is of interest in that in only six cases were there 100 or more organisms for each cubic centimeter of urine. In eight cases there were from ten to 100 organisms for each cubic centimeter, and in twelve there were less than ten. In Table 3 the results of the cultures in this group are given in detail.

In fourteen instances streptococci were isolated from the urine; in six the organism was definitely identified as the *Streptococcus lacticus*, in five it was not definitely identified, and in three it was definitely not *Streptococcus lacticus*. Thus of seventy specimens of urine *Streptococcus lacticus* was possibly present in eleven, and definitely absent in fifty-nine (84.28 per cent).

DISCUSSION

The results of our studies emphasize again the futility of obtaining evidence by the method of Langer and Soldin that in any way will help to settle the problem of the mode of infection in pyelitis. The error that creeps in by contamination, even when all precautions are taken, is such that the evidence obtained is always open to criticism. Our data show that in three-sevenths of the specimens examined the urine was sterile; in two-thirds of these the determination was made in duplicate. We cannot agree with Kleinschmidt that at each catheterization a different flora is obtained, nor with Langer and Soldin that a sterile specimen is only an accident, and repeated catheterization will always yield

bacteria in the urine. We have recatheterized repeatedly and obtained identical results both with regard to sterility and flora. Furthermore, we wish to call attention to types of organisms constantly found by Kleinschmidt and by Langer and Soldin, namely, those of the colon group and *Streptococcus lacticus,* all inhabitants of the intestinal canal and so far as we know not present normally in the urethra. Kleinschmidt asserts that by taking smears from the meatus after cleansing it he was able to prove the constant presence of an organism of the same type as that found in his cultures of the urine. The method used by Langer and Soldin of obtaining cultures is also open to criticism since by washing out the urethra, organisms may easily be washed into the bladder. Langer and Soldin found long chains of streptococci, as shown in one of their illustrations. These they believe have grown from organisms coming from the kidney. The more plausible interpretation is that they are washed in from the urethral opening and are essentially contaminations, as it is not likely that organisms would grow so rapidly in the urine and not grow in more favorable culture mediums after inoculation. Our findings are further at variance with those of Kleinschmidt and of Langer and Soldin in the frequency of occurrence of the *Streptococcus lacticus,* which was almost omnipresent in their cultures. The *Streptococcus lacticus* was definitely identified in only six instances, it may have been present in five others, so that at most it was present in 15 per cent. of the urines examined. In six of eleven instances the *Streptococcus lacticus* was found in the liquid medium and not recovered on the solid medium.

CONCLUSIONS

1. The urine of infants is sterile on culture in from one-third to one-half of the experiments.

2. *Streptococcus lacticus* is only exceptionally found in the urine of infants.

3. The chances of contamination are so great that the presence of organisms in the urine does not prove, without further control, that they have come from the kidney.

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FIVE HUNDRED AND THIRTY FIVE NORTH DEARBORN STREET
CHICAGO

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AN ASEPTIC METHOD OF INTESTINAL ANASTOMOSIS

An Experimental Study

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In consequence of the lack of a satisfactory aseptic method (1) of intestinal anastomosis, and more especially because of the mortality from infection following open resection and suture of the large intestine, the present study was undertaken.

HISTORICAL

Methods and devices have been reported, which are intended to decrease the amount of soiling at the time of operation. However, it may be said that practically all of them necessitate the withdrawal of some instrument or material through the line of sutures in order to complete the anastomosis. One may mention for example, Frank's coupler (2) 1896–1899; the forceps of Laplace (3) 1898; O'Hara (4) 1900, Moszkowitz (5) 1908, and Gudin (6) 1916; Lee's holder (7) 1901; the release sutures of Pockhammer (8) 1906, Wullstein (9) 1908, and Grey (10) 1918; the elastic ligature of Silvestri and McGraw (11) 1891; and the elastic ligature and the gliding metallic draw-string of Pockhammer (8) 1906, and Werelius (12) 1906. Certain procedures have been advanced that are evidently not aseptic, but which facilitate making the anastomosis and in this way somewhat diminish the total amount of soiling. Among these may be included the Murphy metallic button (13) 1892, Abbe's catgut rings (14) 1892, Robson's (15) 1893 and Allingham's (16) 1895 decalcified bone bobbins, Halsted's inflated rubber cylinder (17) 1898, Harrington's (18) 1902 and Gould's (19) 1904 segmented rings with screw handle, Senn's bone plate (20), and other potato-, turnip-, carrot-, bread-, candy-, fibrin-, metallic-, and bone-plates, rings, buttons, cylinders, etc.

The bulkhead method, described by Dr. William Stewart Halsted (21) in 1910 and 1912, was perhaps one of the first more nearly to approach an aseptic operation. Recently (1921 and 1922) Dr. Halsted (1) reported the procedure of abutting the closed ends of the large intestine and puncturing the double diaphragm with an instrument passed per rectum.

In 1916–1917 one of us (Burket) studied various ways to lessen the amount of soiling which occurs during intestinal anastomosis. A wire loop, similar to a tonsil snare, was used to cut through the invaginated stumps of the abutted, closed ends of the intestines. This required the withdrawal of the wires through the line of sutures, and also left a long turned-in cuff of intestinal wall. When the wire was pulled out, the submucosa was occasionally dragged through the suture line and into the metallic tube through which the wire loop worked. Later an instrument was devised, which consisted of a series of knife blades, united to form a diaphragm (like the kodak iris diaphragm), which could be closed by lateral pressure. However, this instrument was difficult to maintain in position, as inserted, and also left a long flap of intestinal wall hanging within the lumen. Various release sutures were studied, but they also necessitated turning in too much intestinal wall and withdrawal of material through the suture line.

Subsequently we have developed an aseptic method of intestinal anastomosis which is dependent for success upon an instrument (Fig. 1) formed of reciprocal male and female halves.

DESCRIPTION OF INSTRUMENT

The male half of the instrument consists of a cylindrical knife blade *a* (Fig. 2), mounted at its back on a central shaft, which is threaded on the portion within and in front of the blade, in order to hold the ratchet of the female tube. A small button *a3*, to serve as a handle with which to rotate the knife, is fastened by the pin *a4* to the rear end of the shaft. The outer surface and back of the cylindrical knife are completely encased in a rigid cover *a2*. The center of the back of this protecting cover is perforated by the shaft and near the center is fastened a small rod, which extends forward through the inside of the cylindrical knife. A spring ratchet is attached to the front end of the rod.

The female half of the instrument is formed of a solid, somewhat conical-shaped structure, *b1*, which contains a projecting central metallic ratchet tube, on the upper surface of which is a metal groove to receive the male protecting-cover rod. Above the groove, within the solid part, is a threaded metallic surface *b3* on which the cover-rod ratchet catches. The female half of the instrument is composed of three distinct

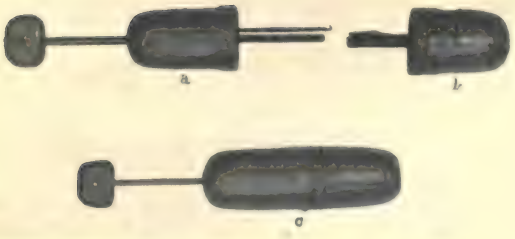

Fig. 1. *a*, Male half and *b*, female half of instrument assembled and ready to invaginate. *c*, Shows the instrument completely reciprocated together and within which only the cylindrical blade is freely movable to be rotated and advanced by means of turning the button on the end of the knife-blade shaft.

segments *b1*, *b2*, and *b3*, which articulate in such a manner that, when assembled, they can be locked immovable by means of a pin *b4*, which perforates the segments from side to side. The segments are essential in order to release the ratchets and to permit easy inspection of the threads, central tube, and groove. A pasteboard disk *b5* covers the flat hard surface of the female half in order to protect the keenness of the circular cutting edge of the male knife blade.

OPERATIVE METHOD OF INTESTINAL ANASTOMOSIS

Our aseptic method of intestinal anastomosis (Fig. 3) is as follows: The intestine is resected between two ligatures of heavy silk, with the cautery knife. Each cauterized end of intestine is invaginated like an appendix stump. The male and female halves (Fig. 1, *a* and *b*) of the instrument described, are inserted one into each gut-end at this time and help to push inward the ligated, cauterized stumps. The two parts of the instrument are so placed that, when they are fastened together, the mesenteric borders will be accurately coapted. Each half of the instrument is held in its respective gut-end by a purse-string suture, which draws a double-walled diaphragm of intestine over each part of the instrument and which is tied down on the projecting central tube, after the instrument has been pushed together just sufficiently to permit the central tube to extend under the edge of the cylindrical blade. It is well, first, to tie the purse-string suture of the female part firmly about the central tube, and then to tie that of the male part loosely around the same tube. After the diaphragms and sutures are carefully inspected to see that all is in the proper position, the halves of the instrument are reciprocated together snugly against the intervening layers of intestinal wall, without

Fig. 2. Instrument unassembled.

traumatizing the gut (Fig. 3). Thus the double diaphragms of the intestinal wall are firmly fixed against the flat surface of the female portion by means of the rigid protecting cover of the cylindrical knife of the male half of the instrument. The intestines are readily anastomosed with interrupted fine black silk sutures. The instrument makes an excellent form on which to take the stitches. It is well to place but not to tie the mesenteric border mattress suture before the two halves of the instrument are ratcheted together, as it is easier at that time accurately to place the stitch, and one can thus be certain of a neat and strong approximation of the mesenteric border. After the mattress sutures have been placed and tied, the operator with his left hand gently grasps the wet cotton-covered intestine which contains the body of the instrument, and with his right hand turns the cylindrical knife by gently manipulating, through the gut wall, the button on the end of the shaft. The button is turned slightly from side to side and moved forward as the knife cuts through the intestinal diaphragms which intervene between its circular edge and the solid surface of the female part. As the knife is the only movable structure within the instrument and the gut layers are firmly fixed against the female block by the protecting cover of the knife, it cuts through the diaphragms and the hand easily detects by feeling when the knife edge reaches the resistance of the hard surface of the female part. Thereupon the instrument, as a

Fig. 3. Diagrammatic longitudinal section; aseptic method of intestinal anastomosis; instrument invaginated into gut ends and reciprocated together ready for intestinal suture and manipulation of cylindrical blade in order to cut out the intervening diaphragms of gut wall.

whole and covered by the invaginated portions of the intestine, slips free and may be slid along out of the gut, or to any desired distance from the field, and permitted to be expelled by the intestines. Thus is promptly re-established the lumen of the intestine, which has not been opened[1] at any time during the operation. The disk of intestinal wall that is excised by the cylindrical blade in order to re-establish the lumen is shown in Figure 4, *a* and *b*. The rent in the mesentery is closed with a running glove suture, care being taken to avoid injury to any blood vessels which may supply the region of the anastomosis. The remaining inturned intestinal collar is just the desired length and, indeed, can be made almost any length by simply turning in more gut wall by means of another anastomotic row of interrupted mattress sutures.

STUDY OF THE ASEPTIC METHOD

We have employed the above method frequently on freshly isolated pig's intestine and also, by aseptic operation, on living dogs under ether anæsthesia.

We have determined that a smooth-edged, solid, circular knife blade (Fig. 2, *a1*) cuts easily, cleanly, and uniformly against a solid surface; while an irregular, serrated, or scallop-edged knife or a series of knife blades seem to be unsatisfactory because almost invariably shreds of submucosa are dragged into the crevices of the blade. These shreds are difficult, finally to divide completely, and hence, complicate the operation.

We have other designs of instruments which work, but thus far the form described in this report is the most satisfactory. A series of different-sized instruments is at hand in order to be adaptable to intestines of varying diameter.

The method is suitable for small and large intestines.

[1] At each site of resection, the intestine is divided by the actual cautery between two tightly placed ligatures and the stumps are thoroughly cauterized during this procedure.

Fig. 4. Specimens of healing anastomosis from dogs that were operated upon by this method; *a1*, healing at end of seven days; *a*, shows the disk of the diaphragm of the abutted intestinal walls that was cut out by the cylindrical knife in order to re-establish the lumen in specimen *a1*, and which passed per rectum (together with the instrument) within 40 hours after operation; *b1*, healing at end of 14 days; *b*, the disk removed by the cylindrical knife from specimen *b1*, and which passed per rectum (with the instrument) within 40 hours from the time of operation.

ADVANTAGES

The advantages of this method of intestinal anastomosis are that—

1. It cuts through the closed abutted ends of the gut and promptly re-establishes the lumen without exposing the operative field to soiling from the opened intestine or by the withdrawal of any material or instrumental device through the line of sutures;

2. No mechanical device is left *in situ* to interfere with the healing (as is the case of the Murphy button). The cylindrical knife serves only as one of the temporary instruments of the operation, just as do the needles and hæmostats, and is removed from the field before the operation is completed;

3. The remaining inturned cuff of intestinal wall is a desirable length, and is neatly, sharply, and uniformly cut;

4. An anastomotic opening is obtained which closely approximates in diameter the gut lumen

and thus removes the opportunity for temporary obstruction, or too great narrowing of the lumen which results from a long inturned collar of intestines;

5. Soiling is reduced to a minimum, dependent upon the effectiveness of the cautery and the skill with which sutures are placed in the submucosa without penetrating the mucosa.

It is intended at a later date to present further studies with our aseptic method of intestinal anastomosis.

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Chronic Infectious Arthritis

STATISTICAL REPORT, WITH END-RESULTS

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It is generally recognized that the end-results of treatment of patients who suffer from a recognized disease entity are a fair measurement of the success of the management and treatment, and also of the correctness of the ascribed cause and of the character of the morbid process. We present herewith the end-results of the treatment of a series of patients with chronic deforming arthritis.

Seventeen years ago, one of us (F. B.) began a study of chronic deforming arthritis (arthritis deformans, rheumatod arthritis) in connection with a clinical investigation of focal infection.

In 1910 it was decided to make an intensive clinical research of chronic deforming arthritis. For this purpose a group of workers was organized, consisting of clinicians, pathologists and laboratory technicians, all of whom were members of the faculty of Rush Medical College and of the staff of the Presbyterian Hospital. The Otho S. A. Sprague Memorial Institute made a financial contribution and repeated it annually for the support of ten beds in the Presbyterian Hospital for indigent patients with the disease. Reports of some of this work have been presented by a few of the workers.[1]

* Read before the Chicago Society of Internal Medicine, Feb. 27, 1922.

1. Billings, Frank: Chronic Focal Infections and Their Etiologic Relations to Arthritis and Nephritis, Arch. Int. Med. **9**: 484 (April) 1912; Chronic Focal Infection as a Causative Factor in Chronic Arthritis, J. A. M. A. **61**: 819 (Sept. 13) 1913; Clinical Aspects and Medical Management of Arthritis Deformans, Illinois M. J. **25**: 11, 1914; Focal Infection, J. A. M. A. **63**: 899 (Sept. 12) 1914; Systemic Diseases of Focal Origin, in Forchheimer's Therapeusis of Internal Diseases **5**: 169, 1914; The Principles Involved in Focal Infection as Related to Systemic Disease, J. A. M. A. **67**: 847 (Sept. 16) 1916; Focal Infection, Lane Medical Lectures, New York, D. Appleton & Co., 1917; Focal Infection, the Oxford Medicine, 1919, New York, Oxford University Press, p. 317. Davis, D. J.: Chronic Streptococcus Arthritis, J. A. M. A. **61**: 724 (Sept. 6) 1913. Jackson, Leila: Experimental Arthritis in Rabbits, J. Infect. Dis. **12**: 364, 1913. Rosenow, E. C.: The Etiology of Articular and Muscular Rheumatism, J. A. M. A. **60**: 1223 (April 19) 1913.

In the decade of 1905 to 1915, 577 patients who complained of arthritis were admitted to the service of Dr. Frank Billings in the Presbyterian Hospital, Chicago. Approximately 85 per cent. of these were admitted during the period of 1910 to 1914 inclusive. One hundred and sixty-six of these patients were classified as having acute arthritis, and are not included in this report. The remainder, 411 patients, suffered from chronic arthritis and constituted the basis of study and investigation, both clinical and statistical. In 1917, a questionnaire was mailed to all of the 411 patients and 309, or 75.2 per cent., made satisfactory answers. The exigencies due to the World War prevented a thorough study and publication of the results of the treatment of these patients at that time. For two years following the armistice, local conditions were such that it was necessary to delay the report. To obtain still more definite information of the results of the management and treatment, a similar questionnaire was sent again in August, 1921. Satisfactory replies were received from 226, or 54.9 per cent., of the patients.

Seventy of the patients were admitted to the hospital only for diagnosis and opinion, and remained from one to three days. One hundred and thirty-four patients were in the hospital from one to three weeks, a time considered sufficient for a thorough study of the condition and for the removal of recognized primary foci of infection. Two hundred and seven of the patients were under observation and management in the hospital for periods varying from four to six weeks, and in a few instances for as long as one or two years.

The statistical tables presented herewith will be made more understandable by a description of the pathology, the morbid anatomy and the principles that guided the management and treatment.

PATHOLOGY

Primarily and fundamentally, the clinical research was based on the probability that chronic deforming arthritis is, as a rule, infectious in character; that the chief etiologic factor is a focus of infection either

single or multiple, usually located in tissues communicating with a mucous surface and sometimes with a cutaneous surface; that secondary etiologic foci are often present in lymph nodes, proximal to primary foci and to infected joints, and that both primary and secondary foci of infection may be the source of bacteremia of varying intensity for a period or for intermittent periods of time.

The result of a bacteremia on the host is dependent on the virulence and also on the number of bacteria in the blood stream. The defensive and offensive powers of the blood and of the other tissues of the host may be sufficient and adequate to overcome the invading pathogenic agents, with no lasting injury to the patient; or the invading micro-organisms may be so virulent or their number may be so enormous in the blood stream that the defensive and offensive forces of the host are overwhelmed with a result that may jeopardize the health or the life of the patient. It must be recognized, too, that the resistance of the host, which is always relative and never absolute, may be further diminished by exhaustion and debility due to physical and mental overwork, starvation, chronic diseases, prolonged exposure to cold and wet, and other factors. Lessened resistance of the host due to exposure to extreme cold or wet, or to excessive fatigue especially, may intensify the virulence of the pathogenic invaders in the primary foci, may promote the occurrence of bacteremia, and may precipitate infection of the systemic tissues of the host. It should be remembered, too, that, with a bacteremia, noninfectious morbid conditions of joints due to faults of posture or weight-bearing, to trauma and the wear and tear of life, may increase the susceptibility to joint infection.

In this study we excluded acute infectious arthritis characterized by purulent exudates in the joint tissues and synovial sac which are caused by virulent strains of the streptococcus, pneumococcus, gonococcus, staphylococcus and other bacteria. Chronic infectious deforming arthritis is not attended with purulent exudates, and, if suppuration occurs in the joint tissues during the course of this disease, it is due to secondary sepsis.

This clinical research was centered on a study of the strains of streptococci isolated from the etiologic

primary and secondary foci and from the infected periarticular tissues and synovial sac and from the joint exudate. The green-producing strains of the streptococcus were very predominant in pure and in mixed cultures. In animal experimentation the green-producing strains showed evidence of varying virulence, but usually were nonpyogenic. The hemolytic strains varied in virulence also; some strains produced purulent exudates in the joints of the intravenously injected animal, while other strains caused arthritis with serous or serofibrinous exudates. The quality of tropism was evident in many strains of the isolated bacteria, as manifested by the apparent affinity for the tissues of the joints, and some strains showed a marked affinity for muscular tissues. In a few patients, mildly virulent strains of gonococci were isolated from the only recognizable primary foci. These nonpyogenic strains caused arthritis with serofibrinous exudate in the intravenously injected rabbits.

This study of the character of the bacteria isolated from the primary foci and from the infected joint tissues and muscles of patients, and their effect on intravenously injected animals, was considered presumable evidence that chronic infectious arthritis is usually caused by strains of streptococci which are usually nonhemolytic and of low virulence, and occasionally by nonpyogenic strains of gonococci. The bacteria reach the joints through the blood stream, and lodge in the small vessels of the periarticular tissues, in the terminal vessels of the subserosa, and in the branches of the nutrient artery which end in the epiphysis. The reaction of the infected tissues is consistent with the degree of virulence of the infectious invaders which may be compared to minute emboli. Clumps of bacteria may block the small blood vessels, or the endothelial proliferation caused by the infection may obstruct the small blood vessels; local hemorrhages occur, and there may be a serofibrinous exudate manifested by edema and swelling of the periarticular tissues, and the synovial sac may contain more or less serofibrinous fluid. The infection may be confined to the periarticular tissues or may be a simple synovitis or, if the infection occurs in the epiphysis of the bone entering into the joint, primary ostearthritis may result. The severity of the

local joint infection and the degree of general systemic disturbance is apparently dependent on the virulence of the invading infectious agents and on the number of bacteria which primarily reach the joint tissues. If the virulence of the micro-organisms is relatively high, the onset may resemble acute rheumatic fever. If the virulence is relatively low, the onset may be insidious and manifested by symptoms of slight involvement of the periarticular tissues only and usually of distal joints. The severe types of infection are usually progressive, and the character of the morbid changes of the joint tissues is hypertrophic or proliferative. The milder types are usually associated with morbid changes of a degenerative or atrophic character.

MORBID ANATOMY

Nichols and Richardson[2] have given an excellent description of the morbid anatomic conditions of the joints in chronic infectious deforming arthritis. They state that the morbid changes, both hypertrophic or proliferative and atrophic or degenerative, cannot be differentiated etiologically. They suggest that the morbid changes are due to the reaction of the tissues excited by toxins derived from infectious invaders or from faulty metabolism and other sources. In this group of patients were individuals who presented all types of arthritis: periarthritis and ostearthritis and synovitis; and individuals also exhibited typical hypertrophic or proliferative changes in one or more joints, while in other joints the morbid changes were atrophic or degenerative. The hypertrophic type was most frequently found in the young and vigorous individuals. Two of the children presented good examples of Still's disease, both typical examples of focal infection. Hypertrophy of bone in the form of Heberden's nodes and hypertrophic or atrophic changes in the larger joints were in evidence in the elderly patients of the group. Several patients presented typical villous arthritis of the knee joint, while in other joints the infection was manifested by milder anatomic forms of synovitis or periarthritis.

The study of this group of patients justifies the view that these morbid tissue changes are probably

2. Nichols, E. H., and Richardson, F. L.: Arthritis Deformans J. M. Res. **16:** 149, 1909.

secondary to the primary reactions of the joint tissues due to the presence of the invaders or their toxins. If the embolic mode of infection is accepted, the tissue reaction includes the blocking of blood vessels by bacterial emboli and by endothelial proliferation with small perivascular hemorrhages and serofibrinous exudate in the tissues and in the synovial sac, with a tendency to subsequent cellular proliferation and secondary sclerosis and contraction. These morbid conditions interfere with the local blood supply and with the nutrition of the infected joint tissues. Repeated doses of the infectious micro-organisms having their source in the primary or secondary etiologic foci will cause renewed and increased damage to the joint tissues. The relative ischemia that results will be followed by malnutrition of the infected tissues. The local morbid condition will be aggravated by the systemic malnutrition and lessened tone of the general circulation from which these patients suffer usually. It is fair to assume that the local malnutrition and morbid changes in the tissues associated with general debility will lead to secondary degenerative changes in the joint structures.

The marked secondary morbid changes which occur in chronic infectious ostearthritis, characterized by the tendency to the formation of new bone, must be due to faulty metabolism. In ostearthritis of the hip, knee, ankle, elbow, wrist, jaw and the spinal column, it is not unusual to find the ligaments converted into bone. This is so pronounced in some cases of ostearthritis of the spine that the bony overgrowth has been likened to the hardened tallow of a guttering candle. The cartilage and the muscle tendons surrounding some joints may become partly or wholly converted into bone. It is difficult to explain the influences which seem to stimulate the conversion of the lower types of these tissues, muscle tendons, ligaments and cartilage into the higher type bone, although the change is explicable on the fact that they have the same mesenchymal origin.

Apparently the part which a deficient blood supply and the consequent poor tissue nutrition may play in the conversion of ligaments, cartilage and muscle tendons into bone was illustrated in some of our patients. One woman, who suffered from a chronic infectious

polyarthritis of moderate severity, wore a cast on one ankle when she was admitted to the clinic. This had been applied three or more months before as an experimental method of relieving the lameness of the ankle. When the cast was removed, the joint was found completely ankylosed. This experience was amplified by the same results of fixation of joints in other patients. It is well known that a joint immobilized by a plaster cast or by other means for a considerable period of time undergoes degenerative changes which may be presumed to be due to arrested function, to poor blood supply and to the consequent poor nutrition of the tissues. Other patients who entered the clinic exhibited one or more joints partly or completely ankylosed, who gave the history of surgical treatment by means of forcible flexion and extension of the joints to correct deformity. The resulting traumatism, added to the existing morbid anatomy, apparently promoted rapid bony ankylosis.

A considerable number of the patients showed diminished carbohydrate tolerance and an increase in the blood sugar. Some patients exhibited moderate blood nitrogen retention associated with slight albuminuria and cylindruria. Digestive disturbance was a common observation, especially of those patients who had suffered from the disease for several years. Coincident involvement of the cardiovascular apparatus was found in many of the patients advanced in years, with the associated degeneration of blood vessels and increase of blood pressure.

CHRONIC INFECTIOUS MYOSITIS

General chronic infectious myositis, with but slight involvement of the joints in the early stages of the disease, was a clinical entity in this group. The morbid tissue changes of the infected muscles was produced apparently in the same manner as the infection of the joints. Examination of the infected muscle tissue of animals intravenously injected with living nonhemolytic streptococci showed embolus-like clumps of bacteria in the small arteries, endothelial proliferation blocking the vessels, and small perivascular hemorrhages. The examination of the muscular tissues of the infected animals one or more months after the experimental injection revealed focal connective

tissue overgrowth, obliteration of small arteries, and a contraction of the muscular bundles due to the connective tissue sclerosis. This histologic examination of the infected human muscle, which was removed under local anesthesia and always with the consent of the patient, showed similar morbid changes characterized by connective tissue overgrowth and contraction.

Some of the patients complained of pain, and this was associated with tenderness of the nerve trunks and of muscle bundles to which the nerves were distributed. This was interpreted as infectious neuritis or perineuritis due to the same infectious micro-organisms.

The deformity of joints in these patients was due to bony overgrowth, to involuntary overaction of flexor muscles to relieve intra-articular pressure, and also to contraction of muscles due to morbid tissue changes caused by infection of the muscles.

CLINICAL COURSE

The onset of the disease in 136 patients was characterized by acute symptoms manifested by acute or subacute arthritis, fever, increase of pulse rate and general discomfort. In 265 patients the onset was insidious. The relation of the onset to the end-result of the management and treatment is shown in Table 9. The clinical study of the group justifies the opinion that the progressive character of the disease is explained by the persistence of the primary or secondary etiologic foci through the failure of their complete eradication. The resulting intermittent bacteremia and reinfection of joint tissues serves to explain the occurrence of exacerbations and the persistence of the morbid conditions which are so characteristic of the disease. The persistence of the joint infection after the spontaneous disappearance of the surgical eradication of the primary and secondary etiologic foci finds explanation probably in the continued presence of the infectious agents in the joint tissues or in tissues which are in situations which do not permit of exploration, as, for example, in the mesenteric and other lymph nodes. After the removal of the etiologic foci of infection, the failure to apply a rational available management and treatment to correct remediable tissue defects explains the failure of improvement or of recovery in some of these patients.

MANAGEMENT AND TREATMENT

The management and treatment of this group of patients was based on the principles that relate to the cause, the mode of infection and the character of the morbid anatomic changes. Primarily, this involved the location and eradication of the apparent etiologic focus of infection. The location of the real focus of infection was difficult occasionally, and at times impossible. The diagnosis and location of the primary focus sometimes required the highest clinical skill and the cooperation of qualified specialists, laboratory investigation and the use of diagnostic instruments of precision, including the roentgen ray. In some patients the failure to eradicate completely the etiologic focus by surgical measures defeated the subsequent management and treatment. The failure to locate the real focus of infection at the beginning was illustrated in some of these patients. Two men of middle age, both of whom exhibited moderately severe periarticular arthritis associated with myositis, showed infected tonsils and associated apical infection of some of the roots of the teeth. These foci of infection were eradicated surgically, and immediate partial recovery resulted. Relapses occurred in both instances, and later both patients developed evidences of calculous cholecystitis. After successful surgical treatment of this infection, both patients made complete recovery. Another patient, a woman, improved for a period after the removal of apparently infected tonsils and the extraction of some infected teeth, and then relapsed into a worse condition. She soon developed classical evidences of acute appendicitis, returned to the hospital, and, after appendectomy, made a prompt and complete recovery. It is probable that the failure to locate and to eradicate the real focus of infection in some of the other patients was the cause of the poor end-result.

The management adopted to overcome the systemic infection, to promote improvement of morbid tissues and to restore function of the joints included the general physical improvement of the patient by the selection of a properly balanced diet, the use of restorative and palliative drugs, attention to personal and general hygiene, a cheerful environment, and the employment of physical therapy, including hydrotherapy, thermo-

therapy, electrotherapy, occupational therapy, mechanotherapy, and regulated passive and active exercise.

While an attempt was made to select a balanced diet, individual peculiarities were considered. Some patients with a low carbohydrate tolerance, others with blood nitrogen retention or with a deficient output of urinary solids due to some morbid kidney condition, and others with manifest defective digestion were given a suitable diet to meet the individual condition. General malnutrition was met by a full, nourishing diet. Some patients were apparently benefited by a deficient but balanced diet over short periods of time. Patients with anemia were given hematinic and other tonics. Pain and soreness of joints and muscles and general discomfort were palliated by salicylic acid compounds. When possible, constipation was corrected by the proper diet and, when necessary, by simple laxatives. Bed rest was maintained during the stage of acute manifestation, and for very painful joints. Painful and sore joints were immobilized by means of splints or plaster casts applied for short periods of time. As soon as the absence of pain and soreness permitted, passive exercise was begun and increased cautiously day by day.

The results of this study emphasize the fact that the primary and fundamental purpose of all forms of physical therapy is to attempt to restore the general and local blood circulation and the nutrition of the tissues, and also the function of the disabled organs. The general debility which was manifested in a majority of these patients was associated with poor general circulation of the blood. The local blood supply of the infected joints and muscles was deficient also because of the general debility of the patient and because of the character of the morbid anatomic changes.

It was found that the improvement of the general condition of the patient by proper diet, rest, environment and other measures aided in the restoration of the tone of the general circulation. The tone of the general circulation was notably improved also by the rational application of hydrotherapy in the form of an alternating hot and cold shower or spray bath under considerable pressure. It was found that this type of hydrotherapy is equivalent in its tonic effects to a

cold plunge bath, and at the same time is devoid of the shock and the unpleasant features of the latter. The circulation of the infected joints was improved also by the local application of the alternating hot-cold spray. Dry heat applied for a short period daily by means of hot air baking, by electric radiant light and by other simple means greatly improved the local blood circulation and at the same time palliated pain and soreness. Diathermy was not used. The proper application of massage, Swedish movements, calisthenics and other active exercises, such as walking, were found to be very helpful at the proper period in the treatment. Occupational therapy suitable for both bed and ambulatory patients was very useful in the restoration of the function of a special group of muscles and, in addition, was a valuable form of diversion to these unfortunates.

The general and special surgeons of the staff cooperated in the correction of deformities by the forcible extension of overflexed extremities by tenotomy and other measures when indicated. The experience gained in the application of forcible movements of the joints, if attended with considerable injury to the tissues by the breaking up of dense adhesions, justifies the opinion that this method of treatment may do more harm than benefit.

The experience gained in this study justifies the opinion that the general improvement of the patient who suffers from chronic infectious arthritis, which will usually follow the rational application of the measures of management and treatment described, will do more to restore the natural resistance of the patient to further local or general infection and to rid the tissues of the infectious invaders, than any other form of treatment. However, in the management and treatment instituted, autogenous vaccines of killed streptococci, a polyvalent horse serum, and nonspecific proteins injected intravenously were used with some of the patients. The autogenous vaccines were made in the laboratory of the hospital and in the laboratory of the John Rockefeller McCormick Institute for Infectious Diseases from strains of streptococci obtained from (*a*) cultures made from primary and secondary foci of infection and from infected muscles and infected joint tissues of patients; (*b*) strains of

streptococci obtained from the joint tissues and muscles of laboratory animals intravenously injected with strains obtained from infected human tissues, and (*c*) autogenous strains *a* and *b* sensitized with the polyvalent horse serum mentioned. These autogenous vaccines were used hypodermically in doses of from 50 millions to 2 billions repeated at intervals at from five to seven days. The effect of the vaccines was studied by noting the local, the general and the focal reaction, and by estimating the opsonic index of the blood of the patient before and after each vaccination. Experience showed that a medium dose of from 300 million to 500 million of the vaccine was quite as effective as larger doses, and this standard dose was finally adopted. The local reaction was usually manifested by slight local tumefaction, occasional slight redness of the skin, and a moderate degree of soreness. The general reaction was almost always objectively negative; subjectively, patients sometimes expressed a feeling of improvement or of greater discomfort. Focal reaction was usually objectively negative, but the patients sometimes complained of greater discomfort in the affected joints or muscles from twelve to twenty-four hours after the vaccination. The opsonic index usually showed an upward curve, but did not disclose evidence of improvement in the immunity of the patient, and after a year of steady application it was abandoned because its value as a measurement of the effect of the vaccination did not justify the great amount of work it entailed.

The polyvalent streptococcus horse serum was made by the intravenous injection of two horses with approximately sixty strains of nonhemolytic and hemolytic streptococci under the direction of Dr. J. J. Moore. Its specificity was tested by an estimation of its contained opsonins. The serum was used in the treatment of eighteen patients by injecting it subcutaneously in gradually increasing doses at intervals of from five to ten days. The local reaction to the injections of the serum was evidenced by a considerable local tumefaction of the subcutaneous tissues, some redness of the skin, and by a considerable soreness, which continued for a day or longer; the general reaction was usually positive, as shown by a slight rise in the body temperature, a moderate increase in the pulse

rate, and a complaint of greater soreness of the joints. The focal reaction was not evident objectively, but virtually all of the patients who received the serum complained of greater discomfort in the infected joints. After one or more injections of the serum, practically all of the eighteen patients became sensitized to the serum, and subsequent doses caused anaphylactic or anaphylactoid shock. The anaphylactic shock was so severe in a few of the patients that it was decided to abandon it because the results of the treatment did not show sufficient benefit to justify the risk which its use involved. The autogenous vaccines sensitized with the polyvalent horse serum did not prove to be of greater benefit than the unsensitized antigen. The intravenous injection of foreign protein, bacterial and others, was usually attended with serious reactions, characterized by chill, followed by high febrile temperature, rapid pulse and great general discomfort. The opposition expressed by patients to the repetition of a second or third treatment restricted its general use. Some patients suffering from the milder types of arthritis were manifestly improved by the intravenous injection of the foreign protein. The small experience with the use of foreign proteins intravenously in this study does not justify an opinion as to the merits of this form of treatment of chronic infectious arthritis.

Of the 411 patients, 229 received autogenous vaccine, eighteen received vaccine and serum, and 164 were treated without vaccines or serum.

STATISTICS

The questions included in the questionnaire [3] were:

1. Have you had any additional focus of infection about the nose, throat, jaws or elsewhere since you were in the clinic?

2. Have you had any marked setbacks in your general condition?

3. (*a*) How much are you able to do in the way of work with the upper extremities? (*b*) How much are you able to do in the way of walking, with or without artificial appliances?

4. What is the condition of your spine?

5. What is the condition of your general health, particularly as regards weight, appetite, bowel movement, etc.?

6. How fully have you carried out the management with particular reference to forms of calisthenic and other exer-

3 Questions 7 and 8 were included only in the questionnaire sent in 1921.

cise, tonic hot-cold-hot bath, avoidance of fatigue and proper food?

7. What treatment prescribed by us or others (exclusive of Question 8) have you had since you last attended the clinic?

8. Have you used with or without your doctor's advice any vaccines, serum, etc., and if so, what kind?

9. General remarks.

TABLE 1.—AGE INCIDENCE AT ONSET OF DISEASE

Age	Males	Females	Total	Osler's Series
1-10	1	4	5	21
11-20	15	29	44	72
21-30	50	62	112	108
31-40	37	58	95	107
41-50	23	53	76	83
51-60	25	35	60	66
61-70	4	15	19	27
71-80	0	0	0	4
Doubtful	0	0	0	12
Total	155	256	411	500

TABLE 2.—AGE INCIDENCE ON ADMISSION TO THE CLINIC

Age	Males	Females	Total
1-10	0	2	2
11-20	6	14	20
21-30	30	42	72
31-40	49	54	103
41-50	28	64	92
51-60	24	53	77
61-70	17	23	40
71-80	1	4	5
Total	155	256	411

The youngest patient was a girl, aged 4 years, and the oldest was a man, aged 68. It is to be noted that the period of greatest incidence was in the third and fourth decades of life; 207 patients (50.3 per cent.) appear in these groups. In 161 patients (39.1 per cent.) the disease occurred before the age of 30 years. For the purpose of comparison, the figures of the incidence of the disease in the series reported by Osler are given in Table 1. It will be noted that 256 of the patients in this series were females and 155 were males. In the 500 patients reported by Garrod, which did not include patients with spondylitis, 411 were females. The group reported by Osler represented 256 females and 244 males. All of these reports indicate that the disease occurs more frequently in the female than in the male sex.

TABLE 3.—DURATION, BY YEARS, OF DISEASE PREVIOUS TO ADMISSION TO THE CLINIC

Age	Males	Females	Total
0-1	28	39	67
1-2	29	37	66
2-3	18	39	57
3-4	16	16	32
4-5	11	25	36
5-6	4	18	22
6-7	4	9	13
7-8	4	10	14
8-9	4	11	15
9-10	5	7	12
10-15	17	24	41
15-20	8	10	18
20 or more	7	11	18
Total	155	256	411

TABLE 4.—OCCUPATIONS REPRESENTED

Indoor Occupations:	Males	Females	Total
Housework	0	195	195
Salespersons (clerks)	35	7	
School (student)	7	12	
Teacher	4	12	
Merchant	14	0	
Manager	8	0	
Nurse	1	7	
Garment worker (seamstress)	2	6	
Stenographer	1	7	
Telephone operator	0	7	
Lawyer	6	0	
Banker	4	0	
Brewer	3	0	
Barber	2	0	
Baker	1	0	
Shoemaker	1	0	
Printer	1	0	
			148
Total indoor occupations			343
Outdoor Occupations:			
Building trades	23	0	
Farmer	18	0	
Physician	13	1	
Engineer	6	0	
Artist	2	1	
Minister	1	0	
Total outdoor occupations			65
No occupation given	2	1	3
Total	155	256	411

On admission to the clinic, ninety-four patients (22.3 per cent.) were 30 years of age or less. This is in agreement with the statistics of Osler, in which 22 per

cent. were under 30 years of age on admission to the hospital. The greatest age incidence on admission to the hospital was in the fourth and fifth decades.

Table 3 is a more detailed statistical compilation of the information given in Table 2. One hundred and thirty-three patients (32.3 per cent) had suffered from the disease less than two years, on admission to the clinic, while seventy-seven patients (18.7 per cent.) had the disease more than ten years before they were admitted to the hospital.

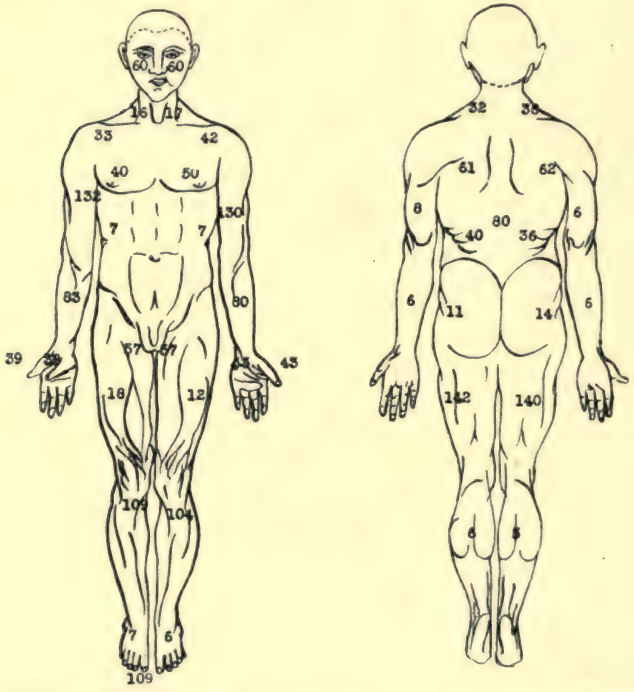

Fig. 1.—Morbid anatomic changes due to true myositis and to spasmodically contracted and atrophic muscles.

The possible contributory etiologic influence of occupation is shown in Table 4. It will be noted that the 195 women patients (47.4 per cent.) were engaged in some form of general housework. One hundred and forty-eight male and female patients (36 per cent.) were engaged in some other form of indoor occupation, making a combined total of 343 (83.4 per cent.) of the

411 patients who were engaged in indoor occupations. Sixty-five patients (15.6 per cent.) were engaged in outdoor occupations, and it is of interest to note that of these, fourteen (3.4 per cent.) were physicians. In the series reported by Osler, a large majority of the patients had been engaged in indoor occupations.

The attempt to classify the patients on the basis of the apparent anatomic changes is shown in Table 5. It will be noted that 311 patients exhibited the mixed types of the disease. One hundred patients were fairly

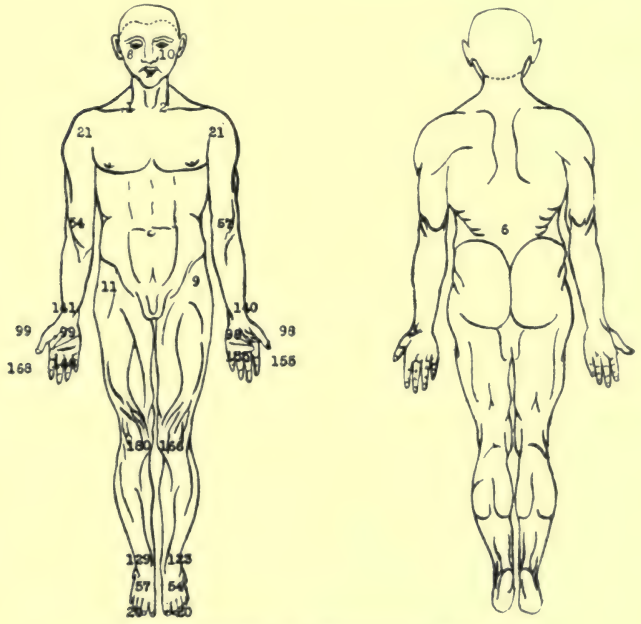

Fig. 2.—Incidence of periarthritis in the joints.

representative of pure types of either ostearthritis, periarthritis or myositis. Not all of the thirty-four patients classified as myopathic were free entirely of some joint involvement, but the incidence of myositis was predominant.

The classification of the disease based on the morbid anatomic conditions serves a useful purpose in a clinical description of the disease. This study of the group of patients indicates that the several degrees of morbid

anatomic conditions recognized in such a classification are more properly stages of the development of the infectious process in the joints. The element of time in which the disease has existed must be taken into consideration in any attempt to classify the disease on an anatomic basis.

In Figure 1, the numerals indicate morbid anatomic changes of muscles due to true myositis and also to spasmodically contracted and atrophic muscles.

TABLE 5.—TYPE OF INVOLVEMENT

Mixed:		
Predominant myopathic	58	
Predominant periarthritic	208	
Predominant ostearthritic	45	
		311
Virtually Pure:		
Myopathic	34	
Periarthritic	55	
Ostearthritic	11	
		100
Total		411

TABLE 6.—PROBABLE FOCI: HOW OFTEN AND IN WHAT COMBINATIONS THEY WERE FOUND

	No.		No.
None found	25	Tonsil and apendix	1
Tonsil only	205	Tonsil, uterus and tube	6
Tonsil and teeth	97	Teeth only	27
Tonsil and sinus	4	Teeth and sinus	1
Tonsils, teeth and sinus	4	Teeth and pus tubes	1
Tonsil and bronchi	1	Teeth and prostate	1
Tonsil, teeth and bronchi	2	Sinus only	1
Tonsil, teeth, bronchi and sinus	1	Prostate and genito-urinary tract only	12
Tonsil and prostate	9	Uterus and appendages only	4
Tonsil, teeth and prostate	2	Middle ear only	1
Tonsil and gallbladder	2	Bronchi only	1
Tonsil, sinus and gallbladder	1	Enterocolitis	1
Tonsil and enterocolitis	1		
Total			411

In Figure 2, the numerals indicate the incidence of periarthritis of the respective joints. Attention is directed to the fact that spondylitis of periarthritic type was encountered in six patients.

In Figure 3, the numerals indicate the incidence of ostearthritis, which includes twenty-six patients with spondylitis.

It will be noted, in Table 6, that no primary or secondary focus of infection was found in twenty-five

patients. As stated previously, there exists always the possibility that a focus of infection may exist in some organ or tissue inaccessible to methods of examination. Table 6 indicates the site of single or multiple foci of infection in 386 patients.

Table 7 indicates the number of times that a primary or secondary focus of infection was found in designated sites.

In connection with the statistics of the incidence and the location of primary foci of infection, it should be added that, as to the previous existence of foci of infection, evidenced by the histories obtained at the time of

TABLE 7.—NUMBER OF TIMES EACH FOCUS WAS CONSIDERED A PROBABLE SOURCE OF INFECTION

	No.		No.
Tonsil	336	Prostate and genito-urinary tract	24
Teeth	136	Gallbladder	3
Sinus	12	Enterocolitis	2
Bronchi	5	Appendix	1
Uterus and tubes	12	Middle ear	1

TABLE 9.—RELATION OF TYPE OF ONSET TO END-RESULTS; BASED ON 1917 RETURNS

	Worse or Unimproved	Improved	Practically Recovered or Cured	Dead	No Report	Total
Acute onset	23	42	30	4	37	136
Insidius onset	53	97	39	12	64	265
No record	3	3	2	1	1	10
Total	79	142	71	17	102	411

admission of the patients to the clinic, forty-four patients (10.7 per cent.) denied knowledge of previous focal infection. Two hundred and twenty-one patients (53.7 per cent.) gave a definite history of attacks of tonsillitis. Seventy-two patients (17.5 per cent.) had suffered from attacks of rheumatism, and forty of these patients presented evidences of valvular defects of the heart. Thirty-five patients (8.5 per cent.) had suffered from dental infection. Seventy-four (17.6 per cent.) confessed to previous genito-urinary infection.

The preponderance of nonhemolytic types of streptococci is shown in the statistics as made from the infected excised lymph glands, joint tissues and pieces

TABLE 8.—ORGANISMS OBTAINED

Focus of Infection	No Streptococci Found	Not Removed, No Record, or Removed Before Admission	Only Streptococci Found Were Hemolytic Streptococci	Only Streptococci Found Were Nonhemolytic or Green Producing Streptococci	Mixed Streptococci: Nonhemolytic Predominating	Mixed Streptococci: Hemolytic Predominating	Gonococci Only	Mixed Gonococci and Streptococci	Staphylococci Only	Total
Tonsil	0	82	20	34	150	50	0	0	0	336
Teeth	16	29	8	63	39	5	0	0	1	161
Sinus	3	0	1	5	3	0	0	0	0	12
Bronchi	0	0	0	0	4	1	0	0	0	5
Prostate and genito-urinary tract	6	0	2	10	0	1	6	2	3	30
Uterus and tubes	1	2	0	8	1	0	0	0	0	12
Gallbladder	0	2	0	0	1	0	0	0	0	3
Appendix	0	1	0	0	0	0	0	0	0	1
Stool	5	0	1	1	0	0	0	0	0	7
Ear	0	0	0	0	1	0	0	0	0	1
Gland	6	1	1	19	1	0	0	0	0	28
Joint	6	1	1	5	1	0	0	0	0	14
Muscle	1	0	0	7	0	0	0	0	0	8
Bursa	0	0	0	1	0	0	0	0	0	1
Fibrous node	0	0	0	1	0	0	0	0	0	1
Blood	3	0	0	1	0	0	0	0	0	4
Total	47	118	34	155	201	57	6	2	4	624

of muscle and from foci of infection (Table 8). Comment has already been made on the varying degree of virulence which characterized the isolated bacteria.

It is a generally accepted opinion that the more acute the onset of infectious arthritis, the more favorable the prognosis for improvement and cure, and that the more insidious the onset, the less favorable the prognosis for improvement or cure. This opinion is substantiated in

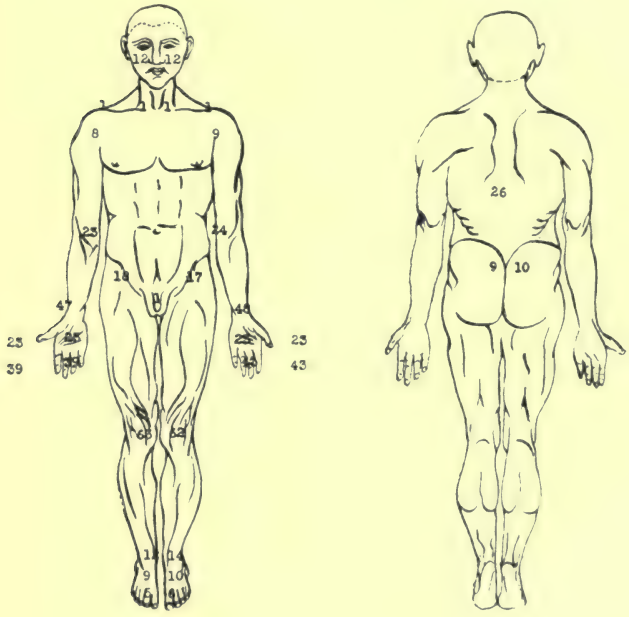

Fig. 3.—Incidence of osteoarthritis.

the statistics of Table 9. Twenty-two per cent. of the patients in whom the disease was manifested by an acute onset recovered, as compared with 14.7 per cent. of the patients who recovered in whom the disease was characterized by an insidious onset, and 16.8 per cent. of the patients manifesting acute symptoms showed end-results which were unimproved or worse, and 20 per cent. of the patients in whom the disease began insidiously remained unimproved or became worse.

It is generally accepted that a more favorable result of treatment of an infectious disease will result if early

treatment is instituted. This principle is especially applicable to the treatment of chronic infectious arthritis. If the treatment and management which has been described is applied to patients who suffer with chronic infectious arthritis before irremediable anatomic morbid changes have taken place, a majority of the patients will recover or, at any rate, will be vastly improved, and the disease may be brought to a standstill. It will be noted that in the group of patients

TABLE 10.—DURATION OF DISEASE IN RELATION TO END-RESULTS, BASED ON 1917 RETURNS

Duration	Worse or Unimproved	Improved	Practically Recovered or Cured	Dead	No Report	Total
Less than 2 years..	12	36	42	4	39	133
More than 2 years.	67	106	29	13	63	278
Total.....	79	142	71	17	102	411

who were admitted to the clinic within two years of the onset of the disease, forty-two recovered, while of the group of patients who were admitted at a period of more than two years of the onset of the disease only twenty-nine recovered.

In estimating the comparative results of treatment with vaccines and serum and without these so-called specific therapeutic agents, it will be noted (Table 11) that 229 patients received autogenous vaccines, of whom eighty-nine patients (38.9 per cent.) of those vaccinated were improved, and thirty-nine (17 per cent.) of those vaccinated recovered; that of eighteen patients who received mixed vaccine and serum, six (33.3 per cent.) were improved, and four patients (22.2 per cent.) of those who received serum recovered; that of 164 patients who did not receive either vaccine or serum, forty-seven (28.6 per cent.) were improved and twenty-eight patients (17 per cent.) who were unvaccinated recovered. These statistics confirm and substantiate the opinion which we have expressed on former occasions that specific remedies in the form of bacterial antigens are of little or no value in the treatment of chronic infectious arthritis.

The importance of physical therapy in the treatment of chronic arthritis is shown in Table 12. Of 186 patients who carried out the management described,

114 (61.2 per cent.) were improved, and sixty-one (32.8 per cent.) recovered. Of ninety-two patients who did not carry out the advised management, twenty (21.5 per cent.) improved and five (5.4 per cent.) recovered. Each patient who remained long enough in the hospital was instructed and educated in carrying out feasible and available types of physical therapy in

TABLE 11.—RELATION OF VACCINE THERAPY TO END RESULTS, BASED ON 1917 REPORTS

	Worse or Unimproved	Improved or Greatly Improved	Practically Recovered or Cured	Dead	No Report	Total
Vaccine.....	55	89	39	7	39	229
Percentage of total cases...	13.3	21.6	9.4	1.6	9.4	44.7
Percentage of vaccinated...	24.0	38.9	17.0	3.1	17.0	100
Mixed vaccine and serum...	3	6	4	1	4	18
Percentage of total.....	0.73	1.46	0.97	0.27	0.97	4.4
Percentage of treated cases	16.6	33.3	22.2	5.5	22.2	100
No vaccine or serum.....	21	47	28	9	59	164
Percentage of total.....	5.2	11.4	6.8	2.2	14.3	39.9
Percentage of unvaccinated	12.8	28.6	17.0	5.4	36.0	100
Total.....	79	142	71	17	102	411
Percentage of total.....	16.6	34.6	17.3	4.1	24.8	100

their homes. Emphasis was made of the importance of the daily application of different types of physical exercise and of hydrotherapy when available. Each patient was especially directed to avoid excessive fatigue and to follow periods of exercise with rest in the recumbent posture. Letters were written to the family physician giving the details of the method of management and emphasizing the need and the value of physical therapy daily. In the earlier periods of the work, autogenous bacterial antigens were prepared and sent to the family physician with directions for their use.

The value of the management and of physical therapy in the treatment of these patients was emphasized over and over again by the improvement of patients who returned to the hospital for further management and treatment the second and the third time because they did not improve at home. In practically

every instance the failure to improve at home was found to be due to the failure to carry out faithfully the management described because of indifference on the part of the patient and members of the family and, in some instances, because of the lack of interest on the part of the family physician.

TABLE 12.—RELATION OF MANAGEMENT TO END-RESULTS, BASED ON 1917 REPORT

	Worse or Unimproved	Improved	Practically Recovered or Cured	Dead	Total
Carried out management....	11	114	61	8	194
Did not carry out management.....	68	20	5	8	101
No record.....	0	8	5	1	14
Total.....	79	142	71	17	309

TABLE 13.—END-RESULTS, BASED ON ANSWERS TO QUESTIONNAIRE, 1917–1921

Answers to Questionnaire	Total Answers Received	No Answers Received	Worse or Unimproved	Improved and Greatly Improved	Practically Recovered or Cured	Dead
1917.....	309	102	79	142	71	17
Percentage of total.....	75.2	24.8	16.6	34.6	17.3	4.1
Percentage of answers.....	....	....	25.5	46.0	23.0	5.5
1921.....	225	185	51	44	92	39
Percentage of total.....	54.9	45.1	12.4	10.7	22.2	9.4
Percentage of answers.....	....	....	22.7	19.6	40.5	17.3

Table 13 gives the end-result of the treatment of 309 patients who replied to the questionnaire in 1917, and 225 patients who replied in 1921. In 1917, 142 of the 309 patients (46 per cent.) were improved, and seventy-one (23 per cent.) recovered. In 1921, of 225 patients, practically all of whom were included in the statistics of 1917, forty-four (19.6 per cent.) were improved, and ninety-two (40.5 per cent.) had recovered. It will be noted that in the answers received in 1917, 143 patients (34.6 per cent.) of the total patients of the series were improved, and seventy-one patients (17.3 per cent.) of the total number of patients had recovered.

In estimating the end-results, so far as possible the term "improvement" indicates improvement both anatomic and functional, and the restoration of the patient to the degree which permits of the assumption of life's responsibilities; by "recovery" is meant both anatomic and functional recovery.

CONCLUSION

This clinical investigation confirms and substantiates the present point of view of a majority of clinicians who have had the opportunity to make a careful investigation of chronic deforming arthritis, that it is primarily an infectious disease, and that the infectious micro-organisms which are the cause are usually strains of nonhemolytic streptococci of relatively low virulence, or occasionally strains of nonpyogenic gonococci or even of other bacteria of mild pathogenicity.

The cause of the remarkable transformation of the fibrous tissues which enter into the joint structure and also of muscle tendons, into bone, is an interesting subject for future investigation. If the remarkable results of the animal experiments reported by Oxhausen [4] can be substantiated, it may be possible to apply preventive measures which will obviate these disabling, irremediable secondary morbid changes.

4. Oxhausen, G.: Ueber einfache aseptiche Knochen und Knorpelenkrose, Chondritis desiccans und Arthritis deformans, Verhandl. d. deutsch Gesellsch. f. Chir., Berlin **62**: 40 (Part 1), 446 (Part 2) 1912.

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COAGULABILITY OF THE BLOOD DURING PREGNANCY AND IN THE NEW-BORN *

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The coagulability of the blood has been studied by a number of physiologists and clinicians in recent years. The reason for this is the great practical importance of hemorrhage as a complication of both medical and surgical conditions. Of the various branches of medicine, none has a more frequent incidence or more serious forms of hemorrhage than obstetrics.

The hemorrhages that the obstetrician may be called on to deal with fall naturally into two classes: maternal, and those occurring in the new-born. Every pregnant woman may have antepartum, intrapartum or postpartum hemorrhage. The blood loss varies from the few hundred cubic centimeters that are lost after every delivery to serious bleedings that may cause a fatal termination in a few minutes. The frequency of bleeding sufficiently severe to be termed a pathologic loss of blood is variously estimated by different authors, but it is safe to say that such bleeding occurs in about 3 per cent. of all cases. The amount lost varies from 500 c.c. to 2 liters or more. The pathologic significance of such blood loss is, according to Williams,[1] slight in the majority of cases, for he believes that the pregnant woman is protected in some obscure way against the deleterious effects of the hemorrhage which, in the nonpregnant woman, would result in grave symptoms. However, he cites cases in which relatively small hemorrhages gave rise to extremely serious symptoms and even death.

Hemorrhages occurring in obstetric cases may be divided into two groups or classes:

1. In the mechanical group, physical defects or alterations of the tissues give rise to a failure of the usual physical mechanism that checks hemorrhage, although the coagulation mechanism may be perfectly competent. Into this group fall cases of abortion; placenta praevia;

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1. Williams, J. W.: Am. J. Obst. **80**: 1 (July) 1919.

abruptio placentae; ruptured uterus; ectopic pregnancy; postpartum hemorrhages due to atony, primary or secondary, of the uterine musculature, cervical tears, and rupture or varicose veins of the vagina and vulva. The mechanical factor is relatively frequent in the child following an insecure tie of the cord or a circumcision with improper suture or no suture at all, as in the children circumcised by mohls.

2. In the chemical type, the underlying factor is a failure of the blood to clot firmly in the normal physiologic time limits under normal conditions. This type of hemorrhage is seen almost entirely among babies. The cases occur practically always during the first two weeks of life. Bleeding occurs in the form of capillary hemorrhages, and may come from the nasal mucosa, stomach, bowel, urinary tract, umbilical cord or from a circumcision wound. The chemical type of hemorrhage is very rare in the mothers, and I have seen it only in women who have suffered from a severe mechanical hemorrhage which so depletes the elements necessary for coagulation in the blood that the patient continues to bleed even after the mechanical factor has been removed. This accords well with the fact that hemophilia is very rare in the female members of a family, but is transmitted through them to the male members.

This work was undertaken to throw some light on several points:

1. To establish some figures for the coagulation time in normal pregnant and puerperal women and in newborn infants.

2. To determine whether differences exist in the coagulation time of the blood of the mother and fetus at the time of delivery.

3. To determine whether hemorrhagic tendencies in the mother could be detected from a study of the coagulability of the blood at or before the birth of the baby, and a postpartum hemorrhage prognosticated before the bleeding began.

4. To determine whether the coagulation time of the baby's blood at birth would give a clue to the probable occurrence of hemorrhages in the next few days.

5. To determine whether the predisposition to postpartum hemorrhage following ether anesthesia in obstetric cases is due to alterations in the coagulability of the blood.

TECHNIC

The technic used was essentially that of Howell,[2] and was adopted so that the results obtained might be compared with his and with figures obtained by others using his methods. From a study of the literature on coagulation of the blood, one is struck with the multiplicity of methods and their divergent results, rendering the work of the different writers valueless for comparison. The technical difficulties of making accurate determinations by any method are great enough as it is, and the results obtained are relative, not absolute, so that a standard technic is desirable to minimize the discrepancies.

Three factors were determined in these experiments: calcium time, coagulation time and prothrombin time.

The coagulation time was estimated by placing 1 c.c. of whole blood in a tube recently rinsed with physiologic sodium chlorid solution, and tilting the tube to the horizontal every half minute until the tube could be inverted without losing the clotted blood.

The calcium time was estimated by adding 1 c.c. of whole blood to 6 drops of a 0.5 per cent. solution of calcium chlorid and tilting the tubes in the same manner as for the coagulation time, and reading the end point in the same manner.

The prothrombin time was determined by placing 0.5 c.c. of a 1 per cent. solution of potassium oxalate in a centrifuge tube and adding about 4 c.c. of whole blood. This was mixed thoroughly and centrifuged for ten minutes at moderate speed, and the clear plasma was used for the determinations. Seven tubes were rinsed with physiologic sodium chlorid solution and placed in a rack. To Tube 1 was added 2 drops of a 0.5 per cent. solution of calcium chlorid. To Tube 2 was added 3 drops and so on, until the seventh received 8 drops of the calcium chlorid solution. Five drops of the oxalated plasma was added to each tube, and the time and tube noted at which the coagulum first appeared. The end-point was determined by tilting the tubes every half minute, as in the case of the calcium and coagulation time estimation.

The blood was obtained from the mothers by puncture of the median basilic vein, and drawn into a Luer syringe which had been rinsed with physiologic sodium chlorid solution immediately before the blood was drawn. The fetal blood was obtained by puncture of the vein of the umbilical cord, in most cases just

2. Howell, W. H.: Harvey Lectures, 1916-1917.

before or just after the cord was clamped, but in a few cases from a few minutes to half an hour after the delivery of the placenta. Some investigators have used blood from the cut end of the cord, but this is hardly comparable to blood obtained by venipuncture because of the presence of Wharton's jelly and thrombokinase set free from the cut surface of the cord. In all cases the readings were made immediately after the samples were obtained, and an attempt was made to provide uniform conditions as to temperature and moisture to avoid variations in results due to these factors.

TABLE 1.—COAGULATION TIME OF MOTHER'S BLOOD ANTEPARTUM

Case	Coagulation, Minutes	Calcium, Minutes	Prothrombin, Minutes
1	4.5	5	9
2	4	5.5	6
3	4.5	2.5	11
4	5.5	6.5	3
5	4.5	3	5
6	8.5	8.5	5.5
7	3	5	6
8	6	3	4.5
9	5	4	3.5
Average	5	4.5	5.9

RESULTS

From a study of the figures it would seem that the coagulation time of the blood is not prolonged beyond the normal in pregnant, parturient or puerperal women. Table 1 shows the results of tests made on pregnant women not in labor. It is seen that the average coagulation time was 5 minutes, calcium time 4.5 minutes, and prothrombin time 5.9 minutes. These figures are well below the upper limit of 8 minutes set as the coagulation time of normal blood. Indeed, it would seem that a slight increase in the coagulability of the blood occurs in pregnancy.

Table 2 gives the results of the tests made while the woman was in active labor, and in most cases only a few hours or minutes before the birth of the baby. Theoretically, it might be assumed that the marked physical strain of labor, together with the more obscure, but nevertheless significant, changes in the physiologicochemical constituents of the blood at this time, might have a marked effect on the coagulation time. However, it will be seen from the figures that the average coagulation time was approximately the same as in the preceding series, 4.75 minutes. The calcium time was 4.43 and the prothrombin time 3.82 minutes.

These figures are slightly less than those in Table 1; the difference is not striking enough, however, to warrant drawing conclusions.

Table 3 shows the results obtained from the coagulation time determinations on the blood of women during the puerperium and during lactation. The figures indicate that the coagulation time is about the same as for pregnant women and for women in labor. The average coagulation time was 5.3 minutes, calcium time 5.65, and prothrombin time 3.5 minutes.

Table 4 shows the results obtained from the coagulation time determinations on the blood of the new-born babies. The average coagulation time was 4.5 minutes, calcium time 3.75, and prothrombin time 3.5. In this series the blood was obtained from the umbilical cord by venipunture as soon as possible after the delivery

TABLE 2.—COAGULATION TIME OF MOTHER'S BLOOD INTRAPARTUM

Case	Coagulation, Minutes	Calcium, Minutes	Prothrombin, Minutes
1	5	4.5	4.5
2	4.5	4	2.5
3	4.5	4	2
4	6	5	4
5	5.5	6.5	4.5
6	3.5	4	3.5
7	5	6.5	6
8	5	3	3
9	4	6	2.5
10	6.5	5	2.5
11	3	2.5	4
12	3	3.5	7
13	4	2	4
14	7	5.5	3.5
Average	4.75	4.43	3.82

of the baby. In two cases the blood was obtained fourteen and thirty minutes, respectively, after the placenta was expelled. The coagulation, calcium and prothrombin time was not materially altered in these cases.

None of the babies of this series proved later to be bleeders, so that a statement regarding the coagulation time in this class of cases at the time of birth cannot be made. However, the opinion is ventured, and is supported to some extent by the work of Rodda, that even in these cases the coagulation time is normal at birth.

The prothrombin time estimation in some of the cases was very difficult, owing to the fact that while a clot formed in these cases, it was not very firm, and it was difficult to determine just when it had reached its maximum density.

COMMENT

It would seem from a consideration of these figures that the blood coagulation time in women before, during or after delivery is well within the normal limits, and that blood taken during labor, just before the birth of the baby, coagulates a little more quickly.

These results accord very well with those of Myer Solis Cohen,[3] who made his coagulation observations on drops of blood in a Stender dish at constant temperature on a water bath. He found that menstruation and pregnancy caused no change in coagulation, and that the puerperium decreased the coagulability of the blood slightly. He found also that the intake of fluids decreased the coagulability of the blood, and that the coagulation time was prolonged after meals.

TABLE 3.—COAGULATION TIME OF MOTHER'S BLOOD POSTPARTUM

Case	Coagulation, Minutes	Calcium, Minutes	Prothrombin, Minutes
1	3	2	10
2	6	5.5	4.5
3	5	5.5	4.5
4	6.5	7.5	4
5	5.5	6.5	4
6	7	8	2
7	7	6	2.5
8	6	6.5	2
9	3.5	4	3.5
10	4	5	2
Average	5.3	5.65	3.5

Weiss [4] found that the coagulation time in the new-born was less than the average for the adult, as measured by the Wright method, and, as was seen in these experiments, was frequently the same as that of the mother's blood. In some instances it was delayed, but in these cases the coagulation time of the mother's blood was also delayed. He also found that breast-fed babies had a longer coagulation time than those fed on cow's milk. He ascribed this to the fact that cow's milk contains more calcium and magnesium than mother's milk.

Rodda [5] found that the average coagulation time in new-born infants was seven minutes, and ranged from five to nine minutes. His method consists in placing a drop of blood between two watch crystals and rolling a lead shot through the drop till the shot is caught by the coagulum. He noted that the coagulation time was

3. Cohen, M. S.: The Coagulation Time of the Blood as Affected by Various Conditions, Arch. Int. Med. **8**: 684 (Nov. 15) 1911.
4. Weiss, Hugo: Wien. klin. Wchnschr. **23**: 839, 1910.
5. Rodda, F. C.: Determining Coagulation Time of Blood in New-Born, Am. J. Dis. Child. **19**: 269 (April) 1920.

progressively prolonged till the fifth day, and that it returned to normal by the tenth day. This corresponds to the clinical observation that most of the spontaneous hemorrhages seen in the new-born occur from the second to the eighth day.

The addition of calcium to the blood seemed to increase the coagulability, but not sufficiently to be accounted a significant factor in the hemorrhages of the new-born. This agrees with the work of Emmel,[6] who found that the calcium content of pig embryos was in excess of that in adults in the proportion of 7 : 5. He noted also that the presence of bile in the blood of these embryos is the primary factor in the increased coagulation time. This, he found, could be overcome by the addition of tissue juice, which neutralized the action of the bile. He noted that the blood platelet count was about the same in the embryos as in

TABLE 4.—COAGULATION TIME OF BLOOD OF NEW-BORN

Case	Coagulation, Minutes	Calcium, Minutes	Prothrombin, Minutes
1	5.5	4	3
2	5	3.5	4
3	4	3	5
4	4	2	4
5	4	3.5	4
6	3.5	3.5	2
7	4.5	5	2
8	4.5	4	2.5
9	4	4	4
10	5.5	5	4.5
Average	4.5	3.75	3.5

the adult pigs. The clots obtained were loose and of the sliding type, as opposed to those in my series, which were as firm as the clots observed in the adult bloods. The babies in this series were all full-term.

It is seen from a comparison of the coagulation time of the maternal and fetal blood, as shown in Table 5, that at the time of birth there is very little difference. In most cases, if the coagulation is retarded in one, a similar change is noted in the other, though it may be less marked. These facts would argue that there is probably a free exchange, through the placenta, of the substances that enter into the reaction of coagulation. This, in turn, shows why the hemorrhages of the new-born rarely, if ever, occur on the first day of life, but are usually seen about the fourth day.

Postpartum hemorrhages have been observed clinically much more frequently in patients who have been under ether anesthesia than in patients who have not

6. Emmel, V. E.: J. Exper. Med. **31**: 177 (Feb.) 1920.

been anesthetized. This factor was observed in all cases, but no differences in the coagulation time could be detected in the two groups. It is probable, therefore, that these hemorrhages are due to relaxation of the uterine muscle bundles, thus releasing the constriction on the uterine vessels.

TABLE 5.—COMPARISON OF MATERNAL AND FETAL BLOOD

Blood	Case	Coagulation, Minutes	Calcium, Minutes	Prothrombin, Minutes
Maternal	1	3	2.5	4
Fetal	1	3	3	No good clot
Maternal	2	4	4	4
Fetal	2	4	2	4
Maternal	3	5	6.5	6
Fetal	3	3.5	3.5	2
Maternal	4	6.5	5.5	4.5
Fetal	4	5	5.5	4.5
Maternal	5	3.5	4	3.5
Fetal	5	6	7	4.5
Maternal	6	5	3	3
Fetal	6	5.5	4	3
Maternal	7	4.5	4	2.5
Fetal	7	4.5	4	2
Maternal	8	4.5	4	2
Fetal	8	4.5	4	2
Maternal	9	6	4	4
Fetal	9	4	4	4
Maternal	10	3	3.5	7
Fetal	10	4	3	5

CONCLUSIONS

1. The coagulability of the blood in women before, during and after labor, as measured by the estimation of the coagulation, calcium and prothrombin time, is well within the limits of normal blood coagulability.

2. Maternal and fetal blood at the time of birth of the baby have practically the same coagulation time.

3. There is no evidence from this series of experiments that pregnancy tends to produce a hemophilic state in the mothers; hence, postpartum hemorrhage can rarely, if ever, be ascribed to this cause.

4. Fetal blood at birth clots with normal firmness and rapidity, so that subsequent hemorrhagic tendencies cannot be detected from coagulation tests at this time.

5. Patients who took ether exhibited the same coagulation time as those delivered without ether. Postpartum hemorrhages, therefore, which are more common in these cases, cannot be explained on the basis of decreased blood coagulability.

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